Karnal Bunt (*Tilletia indica*) Introduction Via Wheat Contaminants in Conveyances: Mexican Boxcars

Preliminary Pest Risk Assessment

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Introduction

General

This document was prepared by the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) in response to a request from the North American Plant Protection Organization (NAPPO). As part of its goal to achieve harmonization of the phytosanitary regulations of its member countries, NAPPO has undertaken the analysis of the risk posed by wheat as a contaminant of conveyances. This **preliminary** pest risk assessment (PRA) represents the initial step in the analysis. This document should be considered the starting point in the evaluation of this complex issue. The scope of this preliminary assessment is limited to the potential risk of the Karnal bunt fungus becoming established in the United States as a consequence of its introduction via wheat contaminants in Mexican boxcars.

We recognize that other potential pathways exist for the introduction of the Karnal bunt fungus. Natural pathways (*e.g.*, wind blown spores) may, in fact, constitute a greater risk for introduction and establishment of the Karnal bunt fungus. Though deserving of examination, these pathways are not the focus of this preliminary assessment. Because of time constraints and a perceived priority, we have limited the scope of this document to a single type of conveyance- Mexican boxcars. Finally, our charge from NAPPO was to examine the risk associated with **wheat contaminants**. While *Tilletia indica* spores may indeed be present in railcars devoid of any wheat contaminants, teliospores as contaminants were not the focus of this study. Furthermore, wheat is regulated as a commodity by Canada, Mexico and the United States, while current phytosanitary regulations do not provide for the regulation of fungal spores as surface contaminants.

In completing this preliminary PRA, we utilized a number of resources including, but not limited to the following:

- available literature on the Karnal bunt fungus and the disease it causes,
- previous pest risk assessments and analyses of Karnal bunt,
- consultations with scientists from academia, industry, state regulatory agencies, Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), Agriculture and Agrifood Canada, Sanidad Vegetal, APHIS and the Agricultural Research Service of the USDA,
- pest interception records for the United States-Mexico border.

Historical Perspective

Karnal bunt disease, caused by *Tilletia indica* Mitra, has been known since 1931 when it was described in the village in India for which it is named. Karnal bunt was considered a minor disease in India until the 1970's when the combination of susceptible varieties, cultural practices and a favorable climate conspired to make it a serious economic threat. Karnal bunt was first

reported in Mexico in the early 1970's.

In 1983, APHIS promulgated quarantine action that prohibited the importation of wheat and wheat products, except flour, from Mexico. This action was deemed necessary to reduce the risk of introduction of the Karnal bunt pathogen, an event that would result both in a reduction of the yield and quality of U.S. wheat crops and the loss of export markets. An earlier pathway analysis conducted by APHIS (**Appendix I**) had identified wheat contaminants in railcars as a potential avenue for the introduction of *T. indica* into the United States. In 1984, APHIS prepared an analysis of the risk of *T. indica* introduction in railroad cars from Mexico (**Appendix II**). The analysis concluded that double-walled Mexican boxcars constituted a very high risk pathway for introduction of *T. indica* and recommended they be prohibited. A 1988 APHIS PRA (**Appendix III**) concluded that large wheat growing areas in the southwestern United States might be climatically favorable for the establishment of *T. indica*. In 1991, another APHIS PRA (**Appendix IV**) determined that the Karnal bunt pathogen represented a high risk to the American wheat crop and recommended maintaining the Karnal bunt quarantine on all wheat imports. A 1994 economic assessment by APHIS (**Appendix V**) indicated that annual crop losses due to Karnal bunt in Arizona, Texas, New Mexico and California would total between $406,000 and $1,000,000 per year. The same report indicated that annual losses in export markets could total over $57,000,000 for Arizona and Texas alone.

At its meeting in April, 1995, the NAPPO Executive Committee supported a proposal from Mexico that NAPPO undertake a review of the phytosanitary measures that are currently applied to the inter-regional movement of grain within North America. APHIS has been given the charge of performing a PRA of the risk of wheat as a contaminant of conveyances. Of particular interest, is the risk of introduction of *T. indica* via wheat contaminants in Mexican railroad cars. APHIS has been asked to complete this PRA by September 15, 1995.

The PRA Process

APHIS, Plant Protection and Quarantine (PPQ) has been working to develop a science-based, quantitative PRA process. One of our goals was to harmonize PPQ risk assessment procedures with guidelines provided by the European Plant Protection Organization (EPPO), NAPPO, and the Food and Agriculture Organization (FAO). Pest risk assessment is one component of an overall pest risk analysis. The FAO *Guidelines for Pest Risk Analysis* describe three stages in pest risk analysis:

Stage 1: Initiating the process for analyzing pest risk (identifying pests or pathways for which the pest risk analysis is needed)

Stage 2: Assessing pest risk (determining if it is a quarantine pest, characterized in terms of likelihood of entry, establishment, spread, and economic importance)

Stage 3: Managing pest risk (developing, evaluating, comparing and selecting options for dealing with the risk)

APHIS conducts pathway-initiated pest risk assessments at two levels: "Qualitative" and "Quantitative." Qualitative and quantitative assessments are similar in most respects, but in quantitative assessments we examine quarantine pests in greater detail and provide a quantitative assessment of the likelihood of introduction (Steps 5 and 6 below).

There are two general categories of initiating events for pest risk analyses. A pest risk analysis can be either "pest initiated" (*e.g.*, a quarantine pest is discovered in a new area, a pest is intercepted at a port of entry) or "pathway initiated" (*e.g.*, international trade is initiated in a new commodity). APHIS completes seven basic steps in pathway-initiated plant pest risk assessments:

Step 1. **Weediness Potential:** We assess the weediness potential of the imported species.

Step 2. **Pest List:** We compile a list of potential quarantine pests associated with the plant species to be imported (regardless of what plant part is to be imported).

Step 3. **Identify Quarantine Pests:** We identify quarantine pests and determine which of the quarantine pests must be analyzed further. Only quarantine pests selected for further analysis are subjected to steps 4-7 below.

Step 4. **Pest Risk Potential of Selected Quarantine Pests:** We rate the risk potential of each pest with respect to five different risk elements. Criteria for estimating risks based on the risk elements were largely qualitative, but we assign numerical values (0, 1, 2, or 3 points) for each element. The total of the five risk ratings provides a numerical estimate of pest risk potential for each pest.

RE #1: Climate—Host Interaction
RE #2: Host range
RE #3: Dispersal Potential
RE #4: Economic Impact
RE #5: Environmental Impact

Step 5. **Scenario Analysis for Selected Quarantine Pests**

We use Scenario Analysis to conceptualize the events that would have to occur before pests could be introduced with commercial shipments commodities.

Step 6. **Quantitative Risk Assessment on Selected Quarantine Pests**

We use quantitative risk assessment techniques to analyze pests for which we obtained a risk rating of ten or more (*i.e.*, the pest presents moderate or high plant pest risk). We analyze either individual pests or groups of pests with similar biologies.

Step 7. **Risk Management Options:** The processes of risk assessment and management are interrelated. However, the scope of this document is limited to the risk assessment process. We nevertheless may make brief comments on options for managing risks associated with the requested importations.

This Karnal bunt assessment is unique in that it is not strictly a "pest-initiated" nor a "pathway-initiated" PRA. A single pathway (conveyances, particularly, Mexican boxcars) and a single pest (*T. indica*) are to be analyzed. Consequently, steps 1-4 are not required for this PRA, since the pest is already identified as a quarantine pest. However, since previous PRAs of *T. indica* did not employ the same criteria as those above in determining the Karnal bunt pathogen's potential as a quarantine pest, we have included a sample calculation of its Pest Risk Potential below.

Estimates of Pest Risk Potential (PRP)

For each risk element (see below) a pest is assigned a risk value of **high** (3 points), **medium** (2 points), **low** (1 point), or **not/none** (0 points) as indicated.

The lowest possible PRP is 3; pests with RP values of 3-6 are not considered to represent any significant risk, low risk pests have PRP values of 7-9, medium risk pests have PRP values of 10-12, and high risk pests have PRP values of 13-15. The PRP is considered to be a biological indicator of the potential destructiveness of the pest.

Risk Element #1: Climate—Host Interaction

When a pest is introduced to a new area, if host plants are available and climatic conditions are similar to its native area, it can be expected to behave as it does in its native area. The evaluation will consider ecological zonation, interaction between the geographic distribution of the pest and geographic distribution of the host. For this element, risk values are based on the availability of both host material and suitable climate conditions. To rate this risk element, we use the U.S. "Plant Hardiness Zones" as described by the U.S. Department of Agriculture. Risk values were assigned according to the following. Due to the availability of both suitable host plants and suitable climate,

the pest has potential to establish a breeding colony:

High (3):	In four or more plant hardiness zones.
Medium (2):	In two or three plant hardiness zones.
Low (1):	In only a single plant hardiness zone.
None (0):	In none of the plant hardiness zones.

Risk Element #2: Host range

The risk posed by a plant pest depends on both its ability to establish a viable reproductive population and its potential for causing plant damage. We assumed risk is correlated positively with host range. For pathogens, risk is more complex and depends on host range, aggressiveness, virulence and pathogenicity. For both arthropods and pathogens, we rated risk primarily as a function of host range as follows:

High (3):	Pest attacks multiple species within multiple plant families.
Medium (2):	Pest attacks multiple species within a single plant family.
Low (1):	Pest attacks only a single species or multiple species within a single genus.

Risk Element #3: Dispersal Potential

A pest may disperse after establishment in a new area. Consider the following:

- reproductive patterns in the pest (*e.g.*, voltinism, reproductive output)
- innate dispersal capability of the pest
- whether natural factors (*e.g.*, wind, water, presence of vectors) facilitate dispersal

High (3):	Pest has high reproductive potential (*e.g.*, multiple generations or cohorts per year, many offspring per reproductive event, high innate capacity of a population for increase (*i.e.*, the species is "r-selected"), ***AND*** individuals are highly mobile (*i.e.*, capable of moving long distances — at least 20 km — either under their own power, or by being moved by natural forces such as wind, water or vectors).
Medium (2):	Pest has either high reproductive potential ***OR*** the species is motile.
Low (1):	Neither high reproductive potential nor highly mobile.

Risk Element #4: Economic Impact

Introduced pests are capable of causing a variety of economic impacts. We divide these

impacts into three categories:

1. Lower yield of the host crop (*e.g.*, by causing plant mortality, or by acting as a disease vector)
2. Lower value of the commodity (*e.g.*, by increasing costs of production, lowering market price, or a combination)
3. Loss of markets (foreign or domestic).

High (3):	Pest causes all three types of impacts.
Medium (2):	Pest causes any two of the above impacts.
Low (1):	Pest causes any one of the above impacts.
None (0):	Pest does not cause any of the above impacts.

Risk Element #5: Environmental Impact

Consider the following four elements:

1. Establishment of the pest is expected to cause significant, direct environmental impacts (*e.g.*, ecological disruptions, reduced biodiversity).
2. Pest is expected to have direct impacts on species listed by Federal or State agencies as endangered, threatened, or candidate. An example of a direct impact would be feeding on a listed plant. If feeding trials with the pest have not been conducted on the listed organism (no direct negative data), a pest will be expected to feed on the plant if it feeds on other species within the genus or other genera within the family.
3. Pest is expected to have indirect impacts on species listed by Federal or State agencies as endangered, threatened, or candidate species (*e.g.*, by disrupting sensitive, critical habitat).
4. Establishment of the pest would stimulate control programs consisting of toxic chemical pesticides, or release of nonindigenous biological control agents.

High (3):	Two or more of the above.
Medium (2):	One of the above.
Low (1):	None of the above (it is assumed that establishment of a nonindigenous pest will have at least some environmental impact).

This information is displayed in tabular form with scores for each of the risk elements (see below). The risk potential is estimated by adding together the risk values (one for each risk element).

Pest Risk Potential of *Tilletia indica*

Climate/Host Interaction (0 - 3)	Host Range (1 - 3)	Dispersal Potential (1 - 3)	Economic Impact (0 - 3)	Environmental Impact (1 - 3)	Pest Risk Potential (Total)
3	2	3	3	2	13

Scenario Analysis

Plant pest risk is composed to two general elements, the consequences of introduction of a particular pest and the probability that the pest will be introduced. Our assessment of the consequences of introduction are presented in the calculation of the Pest Risk Potential. The next step is to estimate the probability that the particular quarantine pest would be introduced.

We estimate the probability that particular pests will be introduced as a result of importation in two steps. First, we conceptualize the events that would have to occur before pest outbreaks could occur using the method of **Scenario Analysis**. We then use the results of our scenario analysis to run a series of Monte Carlo simulations to estimate the frequency of pest outbreaks (see next section).

Monte Carlo Simulations

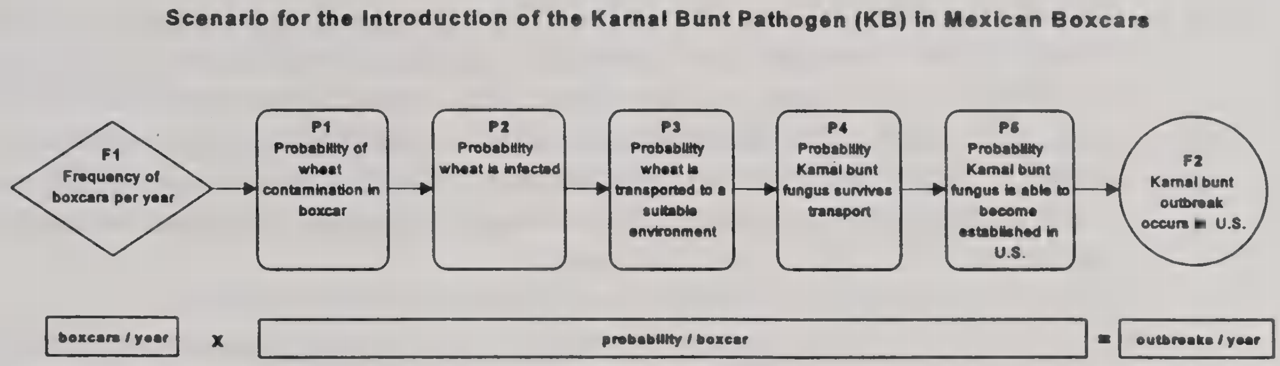
We use the term "Monte Carlo simulation" to refer to the process used to calculate our estimated probabilities of pest outbreaks. The process consists of three steps:

1. Develop a mathematical model to estimate the probabilities of pest outbreaks.
2. Estimate probabilities for each component event in the model.
3. Calculate estimated probabilities of pest outbreaks using Monte Carlo sampling techniques.

Develop mathematical model.

Outbreak probabilities were calculated based on the model shown below. Before a pest outbreak can occur, all of the independent events shown in the model must occur. We use a simple multiplicative model to calculate the estimated frequency of pest outbreaks. To calculate the estimated frequency of pest outbreaks, we multiply the number of boxcars arriving from Mexico per year (F_1) by the probability of the first event (P_1). The resulting product was multiplied by P_2 and so on through P_5. Because the probabilities

are given on a per boxcar basis, the frequency of Karnal bunt outbreaks (F_2) is on a per year basis.



Estimated probabilities for each event in the model.

Because the actual probabilities of the independent events shown above are not known, we estimate them. Although the probabilities are estimated, pertinent data form the basis for the estimate of each independent event. **However, it must be emphasized that these are only estimates with various levels of uncertainty**. We feel the above model is accurate and the level of these uncertainties is reflected in the model's precision (or imprecision.)

F1: Frequency of boxcars per year

In a one month survey conducted by PPQ in 1983, 4,483 boxcars entered the United States from Mexico through the ports of Brownsville, Laredo, Eagle Pass, Presidio, El Paso, Nogales and Calexico (**Appendix VI**). This includes both Mexican boxcars and returning U.S. boxcars. The ports of Roma, Hidalgo, Progresso and San Ysidro had no railcar activity during the survey period. Assuming that the volume of rail traffic during the one month survey was typical, we estimate that 53,796 boxcars per year would cross the border from Mexico to the United States. A three-month survey conducted at Calexico (**Appendix VII**) indicated that approximately 40 percent of the cars crossing the border there were Mexican boxcars. We assumed that the proportion of Mexican boxcars would be similar at other ports. Using these figures, we estimated that the number of **Mexican** boxcars crossing the border would range from 10,000 to 60,000 with a most likely value of 30,000.

P1: Probability of wheat contamination in a boxcar

This estimate addresses only the probability that a railcar is contaminated with wheat and that the wheat contamination is not detected by current port of entry inspection methods. This estimate does **not** address the question of whether the wheat is contaminated with the Karnal bunt fungus.

Factors considered included: the source of the railcar (i.e., has the railcar been used in Mexico to haul wheat); the construction of the car (single wall vs. double); whether or not the cars are carrying cargo; and how likely wheat contamination would be discovered by inspection. Another contributing factor would be the amount of wheat likely to be present in the car. Most experts consulted agree with the assumption made in a previous scenario analysis of the port of El Paso scenario (**Appendix VIII**), that the most likely amount would be about a handful of grain.

The El Paso scenario stated that 32 of 23,789 boxcars entering the United States there were contaminated with wheat. This is a probability of 0.0013. That scenario estimated that the institution of a preclearance program that would allow previously prohibited Mexican boxcars to enter the United States would increase that probability to 0.04.

A three-month survey conducted in 1983 by Calexico PPQ Officer Ted Boratynski resulted in significantly different numbers (**Appendix VII**). During the three month period, 885 boxcars crossed the border at Calexico. More than a third of the boxcars, 348, were Mexican. Wheat contaminants were found in a total of 34 of the 348 Mexican boxcars. Detection rates ranged between about four and eighteen percent. California Department of Food and Agriculture (CDFA) cited similar figures for Calexico in 1984 (**Appendix IX**). Using these figures as a guide, we estimated that the probability of wheat contamination ranged from 0.01 to 0.18 with a most likely value of 0.10.

P2: Probability wheat is infected

This node in the model considers the probability that contaminating wheat in a boxcar will be infected with the Karnal bunt fungus. We used a logic similar to that employed in the El Paso analysis to estimate the probability for this node. We multiplied 30,000 (the most likely number of Mexican boxcars crossing the border) by 0.1 (the most likely probability of a boxcar being contaminated), then by 8 (the number of years for which we had interception records) and divided that number into 151 (the number of *T. indica* interceptions for that eight year period) (**Appendix X**). This value, 0.006, was used as the mean probability for P2. We used a standard deviation of 0.01 and a log normal distribution to provide a range of values that best reflected that predicted by estimates from a panel of Karnal bunt experts.

P3: Probability wheat is transported to a suitable environment

This node estimates the probability that an infected grain of wheat in a boxcar will be transported and deposited in an area that is favorable for the establishment of the Karnal bunt fungus. We only considered whether the environment was favorable for the fungus. Factors to considered included:

> **boxcar route-** it seems likely that once boxcars enter the United States, they will be traveling to many, if not all, parts of the country;
>
> **likely spillage points-** where are contaminating wheat grains most likely to have an opportunity to exit the boxcar (e.g., loading and off loading, bumping, etc.) and would these areas constitute a favorable environment;
>
> **favorable climate-** Karnal bunt does have specific climatic requirements; obvious climatic factors include temperature, rainfall (or irrigation), relative humidity, etc.

Considering these factors, we conservatively estimated the probabilities to range from 0.01 to 0.4 with a most likely value of 0.2.

P4: Probability KB survives transport

The consensus opinion of the expert panel was that there was a high probability that the pathogen could and would survive transport to a suitable environment. Consequently, the values used in the model ranged from 0.6 to 0.9 with a most likely value of 0.8.

P5: Probability KB is able to become established in the U.S.

This node addresses the most difficult question of the entire scenario: given a boxcar contaminated with infected wheat transports that wheat to a suitable environment and the fungus has survived the transit, what is the probability that the fungus will become established in the United States? For the record, FAO defines establishment as, "The perpetuation, for the foreseeable future, of a pest within an area after entry; independent multiplication, completion of life cycle and expected continuation of the species in an area." Using this definition, an infected kernel of wheat that germinates and grows does not necessarily constitute establishment unless and until it spawns a perpetuating population of infected plants. On the other hand, there need not be a Karnal bunt pandemic for the disease to become established. Points considered included:

> **presence of suitable hosts-** *T. indica* has been shown to infect primarily wheat but several other hosts have been identified (**Appendix XI**); what is the likelihood that the infected grain would be deposited where wheat or one of these other hosts

is growing; certain types of wheat more prone to infection (e.g, spring wheat vs. winter wheat, red vs. white, durum vs. club vs. common wheat, irrigated vs. nonirrigated, etc.);

critical mass- the threshold number of infected wheat grains necessary to produce infection;

cultural practices- whether there are cultural practices that either aid or discourage the establishment of *T. indica* (e.g. cultivation, irrigation, etc.).

We estimated that given a boxcar contaminated with infected wheat transports that wheat to a suitable environment and the fungus has survived the transit, the probability that the fungus will become established in the United States had a mean value of 0.001. Because of the complexities of events at this node and the data poor environment within which we were operating, we chose a standard of deviation of 0.01 and a log normal distribution that produced a range of probabilities sufficiently broad to reflect the uncertainty and the variability inherent in this node. We feel that our estimate of 0.001 was probably a conservative one.

Calculate estimated probabilities of pest outbreaks

In the final step of the scenario analysis we calculated the estimated frequency of Karnal bunt outbreaks resulting from infected wheat entering the United States as contaminants in boxcars. The frequency of these outbreaks was calculated as the inverse of the product of the frequency of boxcars entering the United States and the probabilities estimated for each node of the scenario. The frequency of boxcars, in boxcars per year, times the probabilities at each node, in probability per boxcar, yields a product in probability per year, the inverse of which equals the frequency of outbreaks per year.

We used Monte Carlo sampling techniques to account for the uncertainty of estimated probabilities. After choosing probability values, when the Monte Carlo simulations were run (*i.e.*, when the calculations were made to estimate the probability of pest outbreaks), any probability value within the specified limits could be used in the calculations. To increase our confidence that we modeled a sufficient range of reasonable probability combinations, we calculated the final probability 10,000 separate times by running the Monte Carlo simulations with 10,000 iterations. Thus, we obtained 10,000 probabilities of a pest outbreak. The Monte Carlo simulations provide quantitative estimates of the frequency of outbreaks for the scenario and constitute a quantitative risk assessment. We used the personal computer program @*Risk* for Excel (Palisade Corp., Newfield, NY, USA) to run our simulations.

Table 1. Input Data for Monte Carlo Simulation

Node	Distribution	Minimum	Most Likely	Maximum	Mean	Standard Deviation
F1	Triangular	10000	30000	60000	-	-
P1	Triangular	0.01	0.10	0.18	-	-
P2	Log Normal	-	-	-	0.006	0.010
P3	Triangular	0.01	0.20	0.40	-	-
P4	Triangular	0.6	0.8	0.9	-	-
P5	Log Normal	-	-	-	0.001	0.010

Results : Estimated Probability/Frequency of Karnal Bunt Outbreaks

Results of the Monte Carlo simulations are shown in Table 2. Because the probability of pest outbreak was calculated 10,000 times for each Monte Carlo simulation, we present the results by specifying details of the output distribution. The results are presented as the frequencies of outbreaks per year and the number of years between outbreaks (calculated as the inverse of the frequencies). Table 2 presents details of the resulting output distribution (*i.e.*, mean, mode and median of the probability distribution, minimum and maximum values, ninety-fifth percentile value) in terms of the frequency of pest outbreaks per year. By definition, 95 percent of the frequencies generated by the Monte Carlo simulation are less than or equal to the ninety-fifth percentile value. Conversely, five percent of the generated frequencies are greater than the ninety-fifth percentile value. The ninety fifth percentile frequency value is 7.89×10^{-3} times per year or once in 127 years. Consequently, according to our best estimates of the risk of introducing Karnal bunt via Mexican boxcars, we are 95 percent confident that outbreaks will occur less than 7.89×10^{-3} times per year or no more frequently than once in 127 years.

Table 2. Estimates of Karnal Bunt Outbreak Frequency

Description	Outbreak Frequency (per year)	Number of Years Between Outbreaks
Minimum	8.06×10^{-9}	124 million
Maximum	0.92	1.09
Mean	2.59×10^{-3}	386
Mode	1.61×10^{-7}	6.21 million
Median	1.27×10^{-4}	7,870
95% Percentile	7.89×10^{-3}	127

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Subject: Pathway Study

Neovossia indica (Mitra) Mundkur
(=Tilletia indica Mitra)

Common Names: Karnal bunt of wheat
Partial kernel bunt

History
Karnal bunt was first reported from India as far back as 1931. The Department received information on April 26, 1972, from Dr. Norman E. Borlaug, Director of Wheat Improvement, International Center for the Improvement of Wheat and Corn (CIMMYT) that karnal bunt had been found in wheat samples taken from two commercial wheat fields in May 1970 from the Yaqui Valley, Sonora, Mexico. Apparently, this was the first report of the disease in North America.

In July 1981, karnal bunt disease was intercepted in a mail shipment of wheat grain at Laredo, Texas. The wheat seed allegedly originated in Cuidad Obregon, Sonora.

Mexican regulatory officials have recently confirmed that the disease occurred in Mexico (personal communications). Also, it has been documented in the North American Plant Protection Organization (NAPPO) pest list for Mexico dated October 1981.

Damage
Indian scientists report damage to be variable, depending on varietal susceptibility and environmental factors. Crop losses vary from very light to very heavy, depending on the season. There is evidence that the disease is becoming more prevalent and serious in India, particularly on introduced Mexican varieties, but all varieties of wheat in general cultivation in India are found to be affected. Infected seed may germinate but produce weak plants.

Climatic factors
In India, Pakistan, and Afghanistan the disease occurs in the foothills. Infection takes place when conditions are favorable during the flowering period. The optimum conditions for infection occur during periods of high rainfall, high humidity (about 70% RH), and temperatures at 18-22 degrees Centigrade.

Symptoms
The disease is spread by infected seed and soil-borne spores of the pathogen. Infection is restricted to the grain only according to present knowledge of the fungus. Unlike other smuts of wheat, N. indica destroys only part of the grain, the tissue on the grooved side of the kernel, and is thus easily recognized. Dark brown to black spore masses are formed at the base and on the grooved side of the grain, destroying the embryo tissue. Infected grains in the seed head are partially converted into black powdery masses containing smut balls of teuleutospores. Only a few grains in the affected heads of a plant may show the symptoms. In diseased lots, healthy looking seed may be contaminated by teliospores.

Hosts
On Gramineae: *Triticum aestivum* L. (=T. vulgare Vill.) and Triticale

Distribution
Afghanistan, India, Iraq, Pakistan, Mexico, Sweden?.

Pathways
Especially wheat seed; contaminated wheat products such as straw and heads, etc.; bagging used for wheat grain from infected areas railroad cars from Mexico used for transporting wheat seed and wheat products; animal feed containing wheat; soil contaminated with spores, air, packing material, germ plasm.

Karnal Bunt of Wheat
Pest risk analysis: Railroad Boxcars From Mexico

A. Introduction

The objectives of this pest risk analysis are to present—

—the data and information on the Karnal bunt fungus and disease that relates directly or indirectly to the assessment of risk associated with contaminated Mexican double-walled railroad boxcars.

—a risk assessment for boxcars based on biological data.

—a list of options reflecting varying levels of safeguard protection.

—a recommended option to meet the threat delineated by the pest risk assessment.

B. Summary of recommendations

The biological position is that since—

—double-walled boxcars in Mexico become contaminated with teliospores of the Karnal bunt fungus as a result of wheat shipping practices in Mexico.

—there are no inspection and/or treatment safeguard that are both effective and practical to detect or eradicate the fungus in all such boxcars either in Mexico or at the U.S. border.

—teliospores of the fungus could be deposited from these boxcars in wheat growing areas of the United States.

Plant Protection and Quarantine, Animal and Plant Health Inspection Service (PPQ-APHIS) should prohibit the entry of all double-walled boxcars from Mexico regardless of the cargo present at the time of entry.

Management might elect or industry might recommend a less-than-fully-biologically-based position in which case the approach would constitute playing Russian roulette with nature. In Russian roulette, when three events, i.e., (1) a bullet in the chamber, (2) pulling the trigger, and (3) proper aim are synchronized, there is an adverse impact. In playing Russian roulette with nature with the U.S. wheat crop, particularly with exports at stake, when three events are sychronized, i.e., (1) an abundance of Karnal bunt inoculum, (2) the presence of susceptible wheat plants, and (3) favorable environmental conditions at the time of flowering, there would be an adverse impact on the U.S. wheat industry and the segment of the railroad industry involved in moving wheat for export to ports.

If options other than prohibition or exclusion of boxcars were adopted, reliance would be placed on the chances of preventing the syncronization of the host-inoculum-environment interaction. Reliance would be placed on cleaning boxcars by the best available method to reduce the inoculum load knowing that the procedure is not fully effective; on the depositions from boxcars of some spores either outside of wheat growing areas or inside the wheat growing area but relying on the probability that the weather will not be favorable for

infection at the time of flowering. To the extent that the disruption of the synchronization of the three events is prevented, the wheat crop is protected. However, in any year when these events are synchronized, such as in the 1982-1983 growing season in Mexico, an adverse impact would be felt by not only the wheat industry but by the railroad industry if export markets are lost due to Karnal bunt establishment in the United States.

C. Biological data and information relating directly or indirectly to the pest risk assessment for boxcars

Karnal bunt of wheat (KBW) is caused by the smut fungus known in the United States and some countries as Tillitia indica Mitra and in India and some other countries as Neovossia indica (Mitra) Mundkur. The scientific community has not reached an agreement on the scientific name. For convenience, in this analysis, both the disease and the agent will be referred to as KBW.

1. Geographic distribution

KBW is known to occur in India, Pakistan, Afganistan, Iraq, and Mexico. KBW has been intercepted in wheat seed imported into India from Lebanon and Syria, but the occurrence of KBW in these countries has not been reported.

2. Host range

KBW is known to infect common and durum wheat and triticale. For convenience, the term "wheat" in this report will be used to include both wheat and triticale.

3. Potential economic damage

KBW affects wheat production by yield and quality reduction and its presence in the United States would most likely adversely affect exports and the commodity market.

The overall yield reduction by KBW in countries where KBW is known to occur is very low (e.g., less than 0.5 percent) but in some localities the losses range from 1 percent to 40 percent. Usually, KBW appears sporadically but it may become epidemic in some years. For example, in India in an epidemic year, the total damage was 0.3 percent to 0.5 percent but in some fields as many as 89 percent of the kernals were infected.

In addition to a relatively minor effect on yield, quality may be adversely affected when the infection is at the three percent level due to a fishy odor that makes wheat products unpalatable. As of 1983, there had been no reports in the scientific literature concerning mycotoxins present in infested grain. However, a recent unconfirmed report suggests there may be some problem with toxicity when infected grain is fed experimentally to mice.

The greatest impact of the establishment of KBW in the United States would be to adversely affect exports of wheat. The Soviet Union lists KBW on its exotic pest list; China does not specifically list KBW, but China does take regulatory action against Tilletia controversa Kruhn (TCK) even though TCK occurs in that country.

4. Life cycle

A simple diagramatic representation of the life cycle of KBW would show (1) teliospores at or just below the soil surface producing primary or secondary sporidia; (2) the discharge of these sporidia into air currents or the transport of sporidia by splashing water; (3) the landing of sporidia in host flowers, the invasion of ovaries, and then, the developing seed; and (4) the return of teleospores to the soil by the deposit of seeds or teliospores during harvest or threshing.

An understanding of the following characteristics of the fungus and features of its life cycle are critical for the devolopment of a pest risk assessment:

a. KBW does not infect leaf or stem tissue.

b. A seedling developed from an infected seed which is planted cannot be infected by the teliospores or its sporidia (see 4 e). A seedling developed from an infected seed lying on the surface of the ground (e.g., dropped from a boxcar) could be infected if the teliospores germinated during the flowering period and enviromental conditions were favorable for sporidial infection. Volunteer wheat plants or commercial wheat nearby could also be infected.

c. Teliospores germinate when soil moisture and temperature conditions are favorable--unless the spores are dormant. In a given population of teliospores, some teliospores can germinate after a few months of dormancy, but others will remain dormant for at least 4 to 5 years before they germinate.

d. Teliospores produce a promycelium upon which up to hundreds of primary or secondary sporidia are formed. The sporidia are short lived (hours, and apparently, not more than a day). Therefore, sporidia do not contribute to long-distance spread. Teliospores could be blown by the wind, but they are not characterized as airborne in the same sense as sugarcane smut or wheat rust spores.

e. Teliospores which germinate below the surface of the soil at any time or those at the soil surface which germinate before or after flowering produce sporidia which are not available for infecting wheat during flowering.

f. There does not appear to be an efficient means of long distance spread of KBW along natural pathways based on tha analysis of the life cycle of the fungus. The fungus was first reported in the 1950s in India and, during the intervening 30 years, KBW does not appear to have spread over long distances by natural means. The fungus is not known to have spread in any direction from Mexico to other countries since its discovery in 1970. It is hypothesized that the fungus reached Mexico on imported wheat germplasm.

g. Teliospores on infested seed may remain in the soil long after the seed itself has deteriorated. Teliospores deposited on the surface of the soil which have not germinated may be worked into the soil by agricultural operations. Spores from either source may then be worked up to the surface in subsequent years by agricultural operations. These spores, if no longer dormant, could then participate in the infection of wheat by producing sporidia at flowering, provided enviromental conditions were conducive to sporidia infection.

h. PCNB is recommended as a seed treatment since it is the best chemical available. However, PCNB is fungistatic rather than fungicidal (as is true of most fungicides). Consequently, PCNB treatment is not relied upon as the only safeguard. The problem is that PCNB has a half life of up to 1 year depending on soil temperature, but many teliospores have a longevity of at leat 4 to 5 years. If PCNB-treated spores are introduced into the soil at the time of planting PCNB-treated but infected seed, will there be enough active chemical after the first or second year to continue to suppress teliospore germination? Research is under way to develop superior chemical seed treatment.

5. Inspection as a safeguard

a. Field inspection for KBW is not an effective safeguard as symptoms are rarely visible through the glumes. Only when the infection is high and the seed is severely bunted can visual observation detect KBW in the field. The usual symptom, a partial bunt, is not visible in the field.

b. Inspection of a sample of harvested and threshed grain may reveal symptoms, particularly if the seed is examined on a seed-by-seed basis for small lots or by "pan inspection" for larger lots. To the extent that the sample accurately represents a lot, infection may be detected unless the rate of infection is exceptionally low.

c. Inspection can be accomplished by microscopic examination of resuspended pellets obtained by washing the seed sample and centrifuging the washing to concentrate the spores. KBW can be separated from other wheat smuts by this process, but a problem arises if contaminating smuts from other grass species are also pelleted or if a small number of spores are collected. Also, a previously undescribed smut could be present as a contaminant.

If there are no symptoms in the sample, centrifuging may collect contaminating teliospores indicating that the sample has been exposed to KBW. However, detection of KBW by centrifuging, in the absence of seeds with KBW symptoms, is not without problems. Differentiation of KBW from other wheat smuts is based on spore size, surface markings, and, to a certain extent, by color or density. The difference in surface markings can be enhanced by scanning electron microscopy. In addition, research is in progress to determine whether smuts can be differentiated by differences in enzymes.

d. To establish that KBW is present in a country, it is necessary to show infected wheat seeds, with symptoms, from which characteristic teliospores can be develpoed (and, ideally, to then inoculate wheat plants which results in the development of typical symptoms and teliospores). If KBW is detected in centrifuged samples but without typical symptoms symtoms in wheat seed, regulatory emergency action can be taken, if practical, but a search should continue for Karnal bunt symptoms in wheat as a means of confirmation.

6. Treatment as a safeguard

Potential treatments that might be used to eradicate any pest from infested, infected, or contaminated articles or to eradicate hitch-hiking pests not in association with host materials include fumigation, heat treatments, cold treatments, and fungicidal or disinfectant treatments as well as cleaning by washing or vacuuming.

However, none of the known specific treatments in these catagories when applied to KBW are both effective and practical—when practical is defined as operationally or economically feasible—to eradicate KBW from double-walled boxcars imported from Mexico.

It has been demonstrated by PPQ at Calexico, California, that these double-walled boxcars may contain infected wheat seeds or free teliospores which can be recovered from behind the double walls or in the sweepings from the cargo area. Teliospores have been recovered in the air using spore samplers, after boxcars have been swept. Infected seeds have been found on the ground below boxcars. To attempt cleaning all empty cars, or cars which must be first unloaded and then subjected to sweeping and vacuuming may not be feasible. While the cargo area of the car may be cleaned by sweeping or washing, a problem exists in the stirring up of free spores and the drying of the boxcars.

However, infected seeds or teliospores in trash behind the walls cannot be reached by vacuum or even by chemical treatments. Yet, in spite of this difficulty, continued bumping and vibrations or moving cars can eventually dislodge and free seeds or spores for deposit in the United States.

Observations of railroad right-of-ways show volunteer wheat plants that may have developed from farming operations, bird or animal droppings, or boxcars. Often weeds that do not occur elsewhere in the region can be found along right-of-ways or they may spread from the right-of-ways to other parts of the area, suggesting the railroad cars are transporters of seeds.

The following information about treatments relates to KBW sfaguards:

a. Regulatory cold treatments are ineffective. Teliospores can withstand freezing temperatures.

b. Hot water treatment. Teliospores in water can be killed by exposures of 30 minutes to 54°C.

c. Hot air treatments. Dry teliospores are not killed by exposures of 2 hours at 100°C or 3 hours at 75°C. (However, moist spores would be killed by less heat than is required for dry spores, but that data has not been developed) Aeriated steam, often used for soil disinfection and cleaning, would not reach spores nor would it be practical to maintain high enough temperatures.

d. Formaldehyde will kill spores (fungicidal) but the chemical must wet the spores to be effective. Formaldehyde vapors which are known to be a disinfectant in certain other situations are known not to be effective against KBW. Furthermore, formaldehyde is toxic to humans and corrosive to materials. It would not be practical to wet spores or seeds in most areas behind the double walls. Off-loaded cargo placed back into treated cars may take on an undesirable odor.

e. Sodium hypochlorite is effective in exposures for 30 minutes, but the pH of the solution must be adjusted to the alkaline side to release enough chlorine. As with formaldehyde, the solution cannot be expected to reach spores and seeds behind the double walls for an effective exposure period.

f. Vacuuming would reduce the inoculum load in a boxcar, particularly in the

cargo area but would not be effective behind the double walls.

g. High pressure water to wash the boxcars would reduce the inoculum load in the cargo area effectively but would not be effective between the double walls. After washing, it might be necessary to decontaminate the ground where the washing takes place by using the most effective chemicals. However, this too may not be practical.

h. Fungicidal treatments. Most fungicides are fungistatic rather than fungicidal. PCNB is the most effective chemical other than formaldehyde or sodium hypochlorite, but for the reasons discussed in section C, 4, h, it is not considered to be fully effective and, therefore, is used for germplasm as one of a series of independent safeguards. It would be difficult to reach spores and infected seeds behind the interior walls in any event.

i. Although some of these treatments are effective, none are considered as practical because they do not reach behind the double walls, and any cargo must be off-loaded. More importantly, even if any treatment were effective and practical, the time required to handle each boxcar that now enters the United States would be unacceptable to the railroad industry.

D. Risk assessment of Mexican double-walled boxcars

Mexican double-walled boxcars are so constructed that the cargo is held in place by an inner set of wooden walls constructed with boards. An air gap is located between the inner wooden wall and the metal wall of the boxcar. The wooden wall serves to protect the metal wall from cargo shifting and the air gap between walls provides ventilation.

Articles such as seeds, soil, or small pieces of debris, as well as insects, filter through the cracks. Many of the boxcars are employed to move bulk wheat within Mexico, in which case wheat seeds accumulate behind the boards and the accumulation may represent more than one growing season. The 1983-84 growing season for commercial wheat in Senora is projected to have a low severity and low prevalence; consequently, wheat moved in Mexico in 1984 may represent a lower risk because the inoculum load is relatively low. However, the same boxcar may be contaminated with wheat from the 1982-83 growing season when the incidence and prevalence were much higher.

Teliospores and infected grain have been found in boxcars presented for entry at Calexico. Spores were found in the debris of the cargo area and behind the walls. In addition, infected seeds have been found on the ground in a rail yard in California. Volunteer wheat is often found along railroad right-of-ways. Consequently, Mexican boxcars are known to bring seeds and spores to the border from which boxcars now travel significant distances through wheat growing areas.

Inspection and treatment are not adequate safeguards that would result in the detection or killing of teliospores in hidden areas of these boxcars.

Therefore, the only recourse to prevent the entry of these spores into wheat-growing areas is to prohibit double-walled boxcars from Mexico, regardless of the cargo present in the cars. Since it would neither be feasible nor effective to inspect and clean each and every boxcar, it would be necessary to prohibit, in a class action, any and all double-walled box cars.

Although this action may be regarded by the railroad industry as very drastic, it is nevertheless the least drastic action that can be taken.

One could rate manmade pathways on a scale of 1 to 10 with 10 representing the highest risk. The following is a list of some of the pathways and examples of such ratings:

ARTICLE	PEST RISK1/ RATING	BIOLOGICAL REASON	REGULATORY ACTION
Wheat seed from Mexico for planting in the United States.	10	If infected seed is present, inoculum would be placed on or in the soil in a U.S. wheat-growing area.	Prohibit commercial wheat seed; admit scientific seed under permit and a set of independent safeguards which reduce the risk to an acceptable level of 1-2 under risk/benefits consideration
Barley seed for planting	4	Seed could be contaminated with KBW during harvesting or threshing, but barley is not a host; yet U.S. soil could be contaminated by planting barley seed	Proposed action: The same as wheat seed.
Double-walled railroad boxcars from Mexico	9	If infected seeds or spores are present, they could be deposited on the soil surface or near wheat fields or volunteer wheat	Proposed action: Prohibit boxcars since inspection and treatment are not effective or practical.
Used harvesting and combining equipment from Mexico.	7	Equipment could be contaminated with spores or infected seeds.	Proposed action: Prohibit equipment that cannot be cleaned to remove all soil and seeds—and also disinfected.
Wheat straw from Mexico, either raw or processed (e.g. ornamental wheat)	5	Straw could be contaminated with spores or infected seeds	Prohibit wheat straw
Wheat flour from Mexico	1	Processing removes most of spores and all of seeds; intended use in baking eliminates any risk	None
Malted barley from Mexico	less than 1	Seeds have been germinated; material is processed	None

1/10=highest level of pest risk
1=lowest level of pest risk
Scale is based on the concept that there is no "zero pest risk" catagory.

E. Options

Once management has settled on an acceptable level of risk-taking, options for meeting the threat posed by entry of double walled Mexican boxcars can be evaluated as to cost/benefits and risk/benefits. The following options, which are among those which can be considered, are arranged in the order of option with the highest level of protection for use against the highest level of risk listed first, followed by options when the risk is considered to be at a lower level.

If the risk is judged to be high

1. Prohibit all double walled boxcars from Mexico

If the risk is judged to be medium

2. Require cleaning and disinfection in Mexico by the best available methods, even if only partially effective but still feasible. Inspection by Sanidad Vegetal and monitoring by PPQ.

3. Mandatory cleaning and disinfection of all boxcars presented for entry at U.S. points of entry.

4. As in 2 above but only requiring cleaning.

If the risk is judged to be low

5. Various modifications of 2, 3, and 4, but only for boxcars when empty.

6. Allow entry of substantially clean cars based on railroad industry voluntary action.

Virtually no risk

7. Allow entry of all double walled boxcars from Mexico.

Note:

Additional options can be created by various modifications of those presented above. It is beyond the scope of this analysis to present each and every variation but rather to form a framework of options that could be used to develop an operational approach should management not accept option 1 which is the option for high risk.

F. Recommendation

It is the recommendation of the Biological Assessment Support Staff, in particular, and the National Program Planning Staff, in general, that since the movement of Mexican boxcars into the United States is a very high risk pathway for the entry of KBW and, since inspection and treatment are less than adequate as safeguards, such boxcars should be prohibited from entry into the United States as shown in option 1 and in Section B on page 1.

KARNAL BUNT

THE RISK TO THE AMERICAN WHEAT CROP

Robert A. Schall

Program Planning Staff

TABLE OF CONTENTS

I. INTRODUCTION

Karnal bunt, caused by Tilletia indica, was first reported in India in 1931. It remained a minor disease until the 1969-1970 growing season, then it was common in the States of northern India (Delhi, Punjab, Harayana, Rajasthan, and western Uttar Pradesh). In the 1974-1975 growing season the disease was very severe in many places in northern India, particularly in the Tarai region of Uttar Pradesh, Punjab, and Himachal Pradesh. Severity of infection was as a high as 15-23 percent at Hempur in Uttar Pradesh (Joshi et al., 1983).

Karnal bunt was first reported in Mexico in 1972. During 1979-1981 the disease increased to a maximum of 17% infected grains for the cultivar Pima in the Mayo Valley of Sonora (Royer and Rytter, 1985). Both the Mayo Valley and Yaqui Valley in Sonora are infested (Warham, 1986). Recently karnal bunt was reported from the northern part of Culiacan Valley in the State of Sinaloa and in the southern part of Baja California (Hoffman, 1986). These developments indicate that the American wheat crop is threatened by karnal bunt.

To determine the threat to the American wheat crop, this report examines:

(1) environmental conditions that favor development of the disease;
(2) climatic diagrams and homoclimes showing environmental conditions in regions where the disease occurs;
(3) similarity between climatic diagrams and homoclimes for regions where the disease occurs and climatic diagrams for regions where the disease does not occur; and
(4) the likelihood of aerial transport of karnal bunt spores.

II. FAVORABLE ENVIROMENTAL CONDITIONS

Generally the disease is most severe when the temperature is cool, the relative humidity is high, and rainfall is abundant (Aujla et al., 1977; Royer and Rytter, 1985). A examination of the ideal conditions for teliospore (thick-walled resting spore of the smut fungi) germination and sporidia infection during anthesis reveals why these environmental conditions are favorable for disease development.

A. TELIOSPORE GERMINATION

1. TEMPERATURE. According to Zhang and coworkers (1984), the optimal temperature for teliospore germination is in the 15 to 22 C (59 to 72 F) range. According to Smilanick and coworkers (1985), the maximal germination percentages occurred at 15 C (59 F); germination percentages at 20 C (68 F) were slightly lower than those at 15 C.

2. HUMIDITY. Apparently there is no research data giving the optimal relative humidity for teliospore germination. Analysis of the weather data associated with epiphytotics indicates that a relative humidity above 70% is favorable for infection.

3. SOIL MOISTURE. Teliospore germination is arrested by desiccation. However, germination resumes with the return of favorable moisture conditions and achieves levels similar to ideal conditions (Smilanick et al., 1985).

Soil moisture during long-term freezing periods affects teliospore germination. Zhang and coworkers (1984) found that the highest germination percentages occur after freezing in soil with the lowest moisture content (Table 1).

Smilanick and coworkers (1987a) found that teliospores are more likely to persist in dry soils than wet soils (Table 2). Whether buried (6 cm) or at the surface (0 cm), the teliospore germination percent remained high in non-irrigated soil. In irrigated soil teliospore germination remained high only in the buried teliospores. Results of their experiment suggest that rotation from wheat to an irrigated non-host crop is an effective means of reducing soil-borne inoculum.

4. FREEZING. The effect of long-term freezing on teliospore germination is shown in Table 1 and Table 3. One week of freezing had no adverse affect, while two weeks decreased the germination by half. Exposure for two to six weeks gave a fairly constant level of germination. A period of about ten weeks of freezing almost totally inhibited germination, while no teliospores germinated after 12 weeks under dry conditions (Zhang et al., 1984).

In contrast to the research discussed above, Smilanick and coworkers (1985) found that teliospore germination resumed unhindered after a one-week or three-week interruption by freezing at -5 C (23 F). The differences could be produced by the different freezing temperatures (-18 and -5 C; 0 and 23 F) and/or other factors (burial in soil versus application to soil).

Zhang and coworkers (1985) also exposed teliospores to temperatures

fluctuating around 5 and -5 C, 41 and 23 F). Because of contamination, the experiments were difficult to evaluate. However, the teliospores seemed to be able to survive these treatments to a rather high extent. Exposure for 6 weeks followed by germination gave a germination percentage of up to 24% for material kept in 40% soil moisture. In general, no significant difference was observed for the viability of spores kept in soil at various moisture content under fluctuating temperatures.

When experiments on the effect of freezing on teliospore germination are considered, it seems that short-duration freezing (less than one month) at temperatures near 0 C (32 F) has little or no effect on teliospore germination (Smilanick et al., 1985). Short-duration freezing at low temperatures is moderately detrimental to teliospore germination (Zhang et al., 1984; Table 3). Long-duration freezing at temperatures near 0 C seem to be moderately detrimental to teliospore germination (Zhang et al., 1984; flawed experiments). Long-duration freezing at low temperatures is extremely detrimental to teliospore germination (Zhang et al., 1984; Tables 1 and 3).

5. SOIL pH. Teliospores germinate over a wide pH range (6.0 to 9.5) without inhibition. Inhibition occurs below 4.5 and above 10.0 (Smilanick et al., 1985). Therefore, soil pH will not be a factor in teliospore germination.

6. SPORE AGE. Germination of teliospores from freshly harvested kernals was low (6.7%), but increased with storage time (Table 4). By two months the percentage increased to 22.1%; by about four months the percentage increased to 42.3%. After 4 months of storage, germination was only 10% less than that of teliospores stored for 10 to 18 months (Smilanick et al., 1985).

One- and two-year old teliospores show the highest germination, while older teliospore collections show decreasing rates of germination (Warham, 1986). Teliospores can remain viable for five years (Mathur and Ram, 1963).

The position of the teliospores on or in the soil affects viability.

7. SOIL DEPTH. Teliospores that were covered with 2 mm of soil extract agar germinated up to 60%, but promycelia or sporidia did not emere from the agar surface. There was also no evidence of promycelia or sporidia at the soil surface from teliospores covered with 2 mm of soil over a period of six weeks (Smilanick et al., 1985).

In soil experiments with teliospores at 0, 3, and 6 inches below the soil, the maximum length of teliospore survival was 45, 39, and 27 months respectively (Warham, 1986). This increased persistance of teliospores on the soil, rather than under the soil, contrasts with the work of Smilanick et al. (1987a; Table 2). Increased persistance on the soil surface, if it occurs, may be due to reduced soil moisture (and/or reduced biological activity).

The following hypothesis would explain the results of Smilanick et al. (1987a; Table 2). When hosts are not present, persistence on the soil surface may be reduced due to germination of the teliospores under favorable moisture and light conditions. The combination of teliospore germination and lack of infection would reduce the number of viable teliospores. If hosts are present and conditions are favorable for infection, the number of viable teliospores

The information on spore age and soil depth indicates that cultural practices might have a great effect on the severity of the disease. If wheat is only infrequently in the crop rotation and if the soil is moldboard plowed, the inability of aged teliospores to infect when deep in the soil is a factor. If wheat is frequently or exclusively in the crop rotation and if minimum tillage without great disruption of the soil surface is used, teliospores would persist and infect (Smilanick et al., 1987b; Table 2).

Table 1. The influence of deep freezing (-18 C) and soil moisture percentages on _Tilletia_ _indica_ teliospore germination.

Soil Moisture	Teliospore Germination		
	4 Weeks Freezing	8 Weeks Freezing	10 Weeks Freezing
20 %	13.5 %	8.2 %	0.9 %
40 %	9.1 %	7.0 %	0.4 %
80%	5.5 %	4.6 %	0.2 %

Table 2. The influence of irrigation and soil depth on _Tilletia_ _indica_ teliospore germination.

Treatment	Soil Depth (cm)	Percent Germination	
		7 Months (Nov84)	22 Months (Mar86)
Control	-	36.2 a	47.1 b
Irrigated Site	0	46.1 abc	3.0 a
" "	6	60.0 c	1.3 a
Non-irrigated Site	0	43.0 ab	4.6 a
" "	6	50.7 bc	40.4 b

Table 3. The influence of deep freezing (-18 C) under dry conditions on _Tilletia_ _indica_ teliospore germination.

Period of Freezing (Weeks)	1	2	3	4	6	8	10	12
Germination (%)	44.2	21	14.3	13.0	12.2	7.2	1	0

Table 4. Germination of teliospores of *Tilletia indica* after different postharvest storage periods.

Storage Period (Weeks)	Germination (%)
1	6.7
5	17.1
8	22.1
11	35.5
15	42.3
22	47.5

Table 5. Survival of secondary sporidia of *Tilletia indica* at various relative humidities at 25 C.

Relative Humidity	95%	85%	70%	50%	25%
Survival Time (Hr)	12	10.5	8.0	3.5	2.0

B. SPORIDIA INFECTION DURING ANTHESIS

When the teliospore germinates, it produces a promycelium (a short hypha that serves as a basidium). Numerous primary sporidia (the basidiospores of the smut fungi) are produced on the promycelium. The primary sporidia or secondary sporidia, which are produced on the primary sporidia, are carried to the wheat flowers either by air currents or by splashing water. Infection occurs during anthesis (flowering) when the germ tube of the sporidium enters the developing kernal (Bedi et al., 1949; Joshi et al., 1983). Just as there are ideal conditions for teliospore germination, there are ideal conditions for infection by sporidia.

1. **TEMPERATURE.** By measuring the rate of growth of germ tubes of secondary sporidia, Smilanick and coworkers (1987b) determined that the optimal temperature for sporidia was 25 C (5.40 micrometers/hr); growth of the germ tube was similar at 20 C (4.04 micrometers/hr). At higher and lower temperatures germ tube elongation was much less.

2. HUMIDITY. Survival of secondary sporidia was longest (12 hours) at 95% relative humidity. Survival decreased with decreases in relative humidity (Smilanick et al., 1987b; Table 5).

3. RAINFALL. Several researchers have noticed the positive correlation between high rainfall and karnal bunt incidence (Aujla et al., 1977; Royer and Rytter, 1985). Joshi and coworkers (1983) mention the transport of sporidia to wheat flowers by splashing water.

It is also possible that the weather fronts which produce rains also produce winds which distribute the sporidia.

C. DISCUSSION OF ENVIROMENTAL FACTORS

Apparently enviromental conditions will not limit the pathogen from the United States, but will limit the pathogen's geographical distribution within the United States.

Each year wheat flowers early enough to be exposed to cool temperatures in the 15 to 22 C (59 to 72 F) range. Maps (Schlehuber and Tucker, 1967) and a chart (Addendum 1) in the Addenda shows the dates when harvests begin and daily average temperatures for April, May, and June (Environmental Data Service, 1968; Addendum 2). If flowering can be presumed to occur about one month before harvest (Schlehuber and Tucker, 1967), these maps will show the daily average temperature at flowering. The maps indicate that, at least in some years, temperatures will be favorable for floral infection.

In some years there will be moist conditions favorable for the pathogen; in other years the pathogen will survive unfavorable conditions in the teliospore stage. In arid and semiarid areas irrigation will produce suitable moisture conditions for infection during flowering.

Freezing temperatures for long periods, particularly under moist conditions, should prevent the establishment of the pathogen in the northern Great Plains. Apparently the freezing temperatures of the southern Great Plains will have little effect on the pathogen.

The role of tillage is difficult to evaluate. Burial of the teliospores by moldboard plow would prevent the production of sporidia on the soil surface; however, burial would prolong teliospore survival in non-irrigated sites (Smilanick et al., 1987a). There are a variety of tillage systems used in the various regions of the United States (Gebhardt et al., 1985). Presumably, some of these systems will be favorable to the pathogen.

To summarize, the pathogen will flourish in areas with the following characteristics:

1. Cool temperatures during flowering;
2. Moist conditions (through rainfall or irrigation) during flowering;
3. Low soil moisture during the fallow period;
4. Short, mild (or absent) cold period;
5. High nitrogen use; and
6. Repeated planting of wheat with little crop rotation.

Such areas exist in the Southwest.

III. CLIMATIC ZONES, CLIMATIC DIAGRAMS, AND HOMOCLIMES

In the preceding sections the enviromental factors that would produce or influence epiphytotics were discussed. In this section various methods of presenting climatic data will be discussed, because they provide information on enviromental conditions that prevail in a given area. These methods from the works of Heinrich Walter (1973, 1975) are climatic zones, climatic diagrams, and homoclimes.

A. CLIMATIC ZONES. Walter (1973) divides the world into nine main climatic zones. All nine climatic zones will be mentioned, but only those of immediate concern will be described in detail. The climatic zones are:

1. The equatorial zone (I).

2. The tropical zone (II). Lying 10 degree N to 30 degrees N of the equator (and 10 S to 30 S), this zone has a certain seasonal variation in the mean daily temperature. Rainfall reaches a maximum at the time when the sun is at zenith, so that there is a rainy season in the summer and a dry season in the cool months. The duration of the dry season increases as the distance from the equator becomes greater, and at the same time annual rainfall decreases.

3. The subtropical dry zone (III). This zone is poleward of 30 N and S, in the region of the decending air masses, which get warmer as they decend and become very dry. Rainfall is very low, and the daytime temperatures are very high because of intense radiation. In the winter months, however, the temperature may sink to zero at night. This is the hot desert zone.

4. The transitional zone with winter rain (IV). Located at latitudes around 40 degrees, this zone in summer is a high-pressure and dry-air zone, in winter this zone receives cyclonic rain. It has a typical Mediterranean climate with no cold season, but with occasional frost and a long summer drought.

5 - 8. The temperate zones (V - VIII). The temperate zones have cyclonic rain at all seasons which decreases as the distance from the ocean increases. A distinction can be made between a wet, oceanic climate and a dry continental climate with hotter summers and colder winters. The temperate climate regions can be listed as follows:

5. Warm temperate (V). With scarcely any or no winter, this climatic region is extremely wet, especiallly in summer.

6. Typical temperate (VI). Winters in this region are cold, but not too long, or almost free of frost. Summers are very cool when oceanic.

7. Arid temperate (VII). The climate is continental in character, with large temperature contrasts between summer and winter; there is little precipitation.

8. Boreal or cold temperate (VIII). The climate has cool, wet summers and cold winters lasting more than six months.

9. The artic climatic zone (IX).

Addendum 3 is the Commonwealth Mycological Institute (CMI) map showing the distribution of <u>Tilletia</u> <u>indica</u>. According to this 1974 map, this pathogen is definitely found in India, Iraq and Pakistan; a note mentions an unconfirmed report for Afghanistan. According to Joshi and coworkers (1983), the disease occurs throughout northern India from West Bengal, the State in which Calcutta is located, to the western border. The disease was reported in Afghanistan and Mexico besides India, Iraq, and Pakistan; in addition, the pathogen was ... obtained from Lebanon and Syria.

Addendum 4 (Walter et al., 1975) is a map showing the climatic zones in the Asiatic regions where karnal bunt occurs. A comparison of this map with the CMI map shows that in India the disease occurs in the northern portion of the tropical zone, climatic zone II. In this zone there are heavy rains in the summer and drought during the cooler season of the year. In Pakistan where karnal bunt is found, the climatic zones are the tropical zone (II), the subtropical dry zone (III), and a transition zone intermediate between subtropical dry and tropical (II-III). In Iraq the pathogen is found in a subtropical dry zone (III).

Addendum 5 (Walter et al., 1975) is a map showing the climatic zones of North America. Karnal bunt is found in the State of Sonora in northwest Mexico. The Yaqui and Mayo Valleys in Sonora are infested (Warham, 1986); these river valleys appear to be on the border of a transition/subtropical dry zone with summer and winter rainfall (IV-III swr) and a subtropical/subtropical dry zone (II-III).

B. CLIMATIC DIAGRAMS

More precise climatic information can be obtained from climatic diagrams. Addendum 6 is a key to the climatic diagrams; Addendum 7 shows typical climatic diagrams for the nine climatic zones (Walter, 1973).

The climatic diagrams of the areas in India in which karnal bunt occurs resemble the tropical (II) diagram for Salisbury (Addendum 7). Note the rainy season in the summer and the dry season in the cool months.

The climatic diagrams for areas in Pakistan and Iraq in which karnal bunt occurs resemble the subtropical dry (III) climatic diagram for Baghdad (Addendum 7). Note the limited rainfall. At this point the question "How does a pathogen with definite moisture requirements, such as relative humidity, flourish in a limited moisture environment?" must be raised. The answer is that there are two factors that favor the pathogen in an apparently hostile environment. The first factor is that the wheat host is planted to use the cooler periods of limited rainfall, and these periods occasionally have considerable rainfall. The second factor is that the wheat host in the subtropical dry zone is often irrigated; irrigation is associated with disease occurrence (Bedi et al., 1949; Warham, 1986).

Karnal bunt spores have been found in wheat seed shipped from Lebanon and Syria. The climatic diagrams for Lebanon and Syria resemble the climatic diagram for Capetown which shows the transitional zone with winter rain (IV).

C. HOMOCLIMES

Weather stations with similar climates are called homoclimes by Walter (1973). Addendum 8 shows stations throughout the world with numbers indicating their homoclimes. Note the occurrence of homoclime number 146 in India and in Mexico. Also note the occurrence of 159 in India and 160 near the mouth of the Indus in Pakistan and 160 in northwestern Mexico.

IV. CLIMATIC COMPARISONS

The previous sections discussed (1) enviromental factors that favor karnal bunt and (2) climatic information for infested areas. This section will use this enviromental and climatic information to target areas of the United States in which the pathogen could establish itself.

Addendum 9 and Addendum 10 show wheat-growing areas in the United States (Wheat Flour Institute, 1965). These two maps indicate that three wheat-growing areas are likely to be contaminated by air-borne or animal-borne teliospores. The areas are (1) the white wheat area of Arizona, (2) the white wheat area of California, and (3) the hard red winter wheat areas of New Mexico and Texas. These three areas will be discussed in relation to the pathogen-host-enviroment factors that are necessary for disease development.

Due to its proximity to the infested State in Mexico and the flow of the prevailing winds (Environmental Data Service, 1968; Addendum 11), the white wheat area of Arizona is most likely to be contaminated. The first factor for disease development, the presence of the pathogen, is likely to occur, because the teliospore are wind-borne (Warham, 1986) and probably animal-borne (Smilanick et al., 1986). However, development of the disease will depend on two other factors besides the presence of the pathogen. The second factor is the presence of a susceptible host. Duram wheat and triticale are resistant. There are cultivars with resistance, but many cultivars are susceptible or very susceptible (Bedi et al., 1949; Joshi et al., 1983; Warham, 1986). Since hard red winter and hard red spring wheats are also grown besides white wheat (USDA maps, 1969), susceptible hosts are probably present. The third factor is suitable environmental conditions. The climatic diagram for Phoenix (Walter et al., 1975) which is just south of the center of Arizona resembles the climatic diagram for Baghdad (Addendum 7). Because of irrigation and occasionally favorable conditions, the disease can develop under these environmental conditions. Other climatic diagrams, those for Jerome in north-central and Tucson in south-central Arizona, are intermediate between the tropical and subtropical dry diagrams (Addendum 3; Walter et al., 1975). A similar tropical/subtropical dry intermediate zone (II-III; Addendum 4) is locate east of the Indus River; karnal bunt occurs in this region.

The white wheat area of California is also at risk, perhaps as much as the white wheat area of Arizona. Karnal bunt spores have been detected by aerial sampling over Tabasco, which is adjacent to the wheat-growing areas in Arizona and about forty miles from the wheat-growing areas in the Imperial Valley of California. As with the wheat-growing areas in Arizona, this area is likely to be contaminated, because of its proximity to the infested area in northwest Mexico and the comparative ease of teliospore transport. Presumably, susceptible hosts will be present. The climatic diagrams of the white wheat areas of California (Walter et al., 1975) are typical of those of the transitional zone with winter rain (Capetown in Addendum 7). Lebanon and Syria where karnal bunt possibly occurs also have typical transitional-zone climate diagrams.

The hard and soft red winter wheat area of New Mexico and Texas (Addendum 10) is less likely than the preceding area to be contaminated; however, due to the possible transport by migratory insects and favorable winds, this area must be considered at risk. Based on tests of wheat cultivars (Bedi et al., 1949; [illegible] 1983; Royer and Rytter, 1985; Warham, 1986), it is best to

for Clayton in the extreme northeastern corner of New Mexico and Tucumcari in northeastern New Mexico are arid temperate (VII) climate zones. Because the pathogen has the ability to withstand periods of freezing temperature, it is probable that the pathogen can overwinter in spite of the freezing temperatures. There is one factor that may hinder the overwintering of the pathogen; this factor is the presence of high soil moisture levels (Zhang et al., 1984). However, the pathogen may be present in Afghanistan; the arid-temperate climatic diagram for Kabul (Addendum 7) shows a cold season with high moisture levels. If the pathogen is able to overwinter in Afghanistan, it should be able to overwinter in the lower Great Plains.

Zhang and coworkers (1984) believe that the pathogen is easily capable of flourishing in the Yangtze River Valley, a warm-temperate climatic zone (V; Addendum 4) with high soil moisture and a mild winter. In addition, they believe that the pathogen is capable of infecting wheat in the Hwang Ho Valley, a typical-temperate climatic zone (VI; Addendum 4) with high soil moisture and a cold winter. If Zhang and his coworkers are correct, all the soft red winter wheat and much of the hard red winter wheat-growing areas are at risk; compare Addendum 5 with Addendum 10. However, Zhang and coworkers may not give adequate weight to the effect of soil moisture.

V. ANALYSIS OF THE AERIAL TRAPPING OF KARNAL BUNT SPORES

The aerial dispersal of fungal spores has occurred over thousands of kilometers (Pedgley, 1982). Examples of long-distance transport are:

(1) *Hemileia vasatrix* which causes coffee leaf rust was detected in the Bahia state of Brazil in 1970. By 1971, the pathogen was in the Sao Paulo state, and by 1974 in Paraguay and northern Argentina. The spread was in the direction of the dominant wind, and spores were trapped by aircraft over the state of Parana before the disease was detected.

(2) *Puccinia polysora* which causes a maize rust was found in Sierra Leone, West Africa, in 1949. Spreading eastward the fungus crossed West Africa by 1951, crossed East Africa by 1952, and reached Madagascar by 1953. It seems to have spread on the wind during the maize-growing season - when monsoon south-westerlies were blowing over West Africa, and when north-easterlies were blowing over East Africa.

(3) *Melampsora* species which cause poplar rust were detected in New Zealand in March 1973. All information indicates that the spores travelled more than 3000 km from New South Wales during the March 1 to 3 westerlies.

Tilletia indica, the pathogen causing Karnal bunt of wheat, is present in the state of Sonora in northwestern Mexico. This section examines the possibility of long-distance transport of the pathogen to wheat-growing areas of the United States.

A. BASIC PRINCIPLES

According to Aylor (1978) the aerial dispersal of fungal spores involves three highly interdependent events: liberation, transport, and deposition. These events are also known as takeoff, flight, and landing. Quantification of these events permits the calculation of (1) the number of spores deposited and (2) the concentration of spores in the air.

The number of spores deposited per unit leaf area and per unit time is equal to the concentration of spores times the rate of deposition:

Spores Deposited		Concentration		Rate of Deposition
(#/square meter/second)	=	(spores/cubic meter)	X	(meters/second)

The concentration of spores in the air is equal to the number of spores released times the transport characteristics of the atmosphere:

Concentration = Spores Released X Transport Characteristics

Liberation, transport and deposition will be considered in detail to explain the possibilties of long-distance dispersal of the Karnal bunt pathogen to the United States.

B. LIBERATION (TAKEOFF)

Spores can be liberated by wind currents, by the movement of plant parts, by the abrasion of plant parts, and by the puff phenomena associated with the splash of a raindrop impacting on a surface. These are natural phenomena that liberate spores.

In addition, it appears that spores are liberated through the activities of

Addendum 12 present information from aerial trapping of spores before, during, and after burnings of wheat fields. The information is limited; therefore, interpretation is difficult and there exists the possibility of error. Because of the threat posed by the pathogen, the available information will be analyzed in spite of the limitations. This observations also applies to the section on transport and flight.

Prior to the burning of the wheat fields, spores were not found in the air. However, only a single flight was made (June 14, 1988 at Tabasco). To conclude that spores are not present on the basis of a single flight is unsound. It is noteworthy that spore were found the day after burning when it is probable that the infested air mass had travelled away. These spores may have been liberated by natural causes or by burning and may represent an average concentration of spores.

During burning the number of spores increases (Table 6); moreover, the spores are found at exceptional heights. It seems reasonable to state that burning of wheat fields liberates spores (perhaps by the strong convection currents generated) and transports the liberated spores high into the atmosphere. It is noteworthy that the spore concentrations are highest at the highest altitude (possibily with a gradient present). However, there may be a turbulence, eddy, or inversion effect (Waggoner, 1965) near or downwind of the convection updraft.

One day after burning fewer spores were found in the air above the wheat fields (Table 7). This could be due to two factors. One is the sedimentation of spores released during burning. The other is the movement of the spore-infested air mass away from the burning site, and the replacement of the spore-cloud by a representative air mass.

C. TRANSPORT (FLIGHT)

Two factors influence transport. The first factor is the terminal velocity of the spore, that is, the rate at which the spore will fall in still air. The second factor is the movement of the air mass and turbulence within the air mass.

If the air is still, the important physical factor affecting the settling of spores is the terminal velocity of the spore. Terminal velocities for most fungal spores range from somewhat less than 0.1 to nearly 3.0 cm/sec. Terminal velocities for various pollens and fungal spores are in Table 8 (Grègory, 1973). The terminal velocity of the Karnal bunt spore can be appproximated and comparisons made with wind-borne spores and pollens.

According to Gregory (1973), the observed terminal velocity of most spherical and ovoid spores fit the following formula:

$$\text{Terminal Velocity} = \frac{\text{Length X Width}}{40}$$

In the formula, velocity is in millimeters per second and spore dimensions are in microns. With the formula, an approximation of the terminal velosity of Karnal bunt spores is obtainable (Table 9). According to an undated CIMMYT publication, the spore size is 25-30 microns. For 25-micron Karnal bunt spores the terminal velocity would be around 1.5; for 30-micron Karnal bunt spores the terminal velocity would be around 2.2. If the average spore size

is 35 microns in diameter (Waller and Mordue, 1983), the terminal velosity would be around 3.0.

The dimensions of various fungal spores are in Table 10; this information on the wheat pathogens comes from Jones and Clifford (1978). There is little difference in the size of Karnal bunt spores and the easily transported stem rust and leaf rust spores; however, there are differences in the terminal velocities. If the average terminal velosity of Karnal bunt spores is 3.0, this is more than the terminal velosities of the stem rust and leaf rust spores, with respective terminal velosities of 1.06 and 1.26. Therefore, Karnal bunt spores would be less likely to be transported than the mobile stem rust and leaf rust spores which are easily transported long distances (Gregory, 1973).

Large spores with terminal velosities similar to the terminal velosity of Karnal bunt spores are capable of being transported long distances. For example, spores of <u>Helminthosporium</u> <u>sativum</u> have been found in the Northwest Territory of Canada (Gregory, 1973).

Studies of pollen drift supplement those of fungal spores. Many pollens have terminal velosities similar to the terminal velosity of Karnal bunt spores (Table 8) and these pollens are transported long-distances (Gregory, 1973).

If a spore drops 3 cm in 1 sec, it will take 101,600 sec for the spore to drop 3,048 meters (304,800 cm; 10,000 ft). If the spore is being transported in an air mass moving at 5 m per sec (10.8 mi/hr), it will move 508 km (508,000 m) in the time required for the spore to drop.

$$\frac{3\ \text{cm}}{1\ \text{sec}} = \frac{304{,}800\ \text{cm}}{X\ \text{sec}} \qquad\qquad \frac{5\ \text{m}}{1\ \text{sec}} = \frac{X\ \text{meters}}{101{,}600\ \text{sec}}$$

$$3X = 304{,}800 \qquad\qquad X = 508{,}000\ \text{m}$$

$$X = 101{,}600\ \text{sec (28.2 hr)} \qquad\qquad X = 508\ \text{km} = 305\ \text{miles}$$

Measured at 10 meters, the mean wind velosity in England is 5 m/sec . Kew 90% of the hourly means were 1.0 m/sec and upwards; for over half the time the speed was 3.0 sec and upwards (Gregory, 1945). A wind speed of 5 km/sec (10.8 mph) is a typical surface wind speed (Addendum 11), that is, characteristic of the lower boundary layer which is 500 to a 1000 meter in depth. Above the boundary layer, wind speeds are even higher (Pedgley, 1982). Because of the upper-air wind speed, it is appropriate to consider spore or pollen clouds as in suspension in air. At heights of about 1000 meters and upwards the distribution agrees well with that expected from known values of terminal velosity and turbulence (Gregory, 1945).

When spores liberated by the burning of wheat stubble are transported high into the atmosphere and entered an air mass, the movement of the contaminated air mass can be determined by examining wind maps. Wind maps permits the center of an air mass to be located after the elapse of a given period of time (Pedgley, 1982). Computer programs can also be used to track the movement of air masses (Davis 1987; Davis and Main, 1986).

D. DEPOSITION (LANDING)

which the spore was raised, the strength of the wind, the duration of the wind, the presence of downdrafts, the presence of inversions, and/or the washing effect of rain.

If raised to a lower altitude, there would probably be heavy deposition near the source and a rapid falloff away from the source. If raised to a higher altitude, there would likely be a skip distance, a main deposition region, and a tail of reduced deposition (Gregory, 1973).

Besides varying at various altitudes and on individual days, wind strength varies during the day and at night (Pedgley, 1982). The night with its reduced wind and reduced turbulence favors the deposition of spores (Gregory, 1945).

Spore-laden air flowing over horizontal surfaces will deposit spores at rates greater than those calculated for sedimentation under the influence of gravity. Turbulent deposition is the cause of this phenomenon (Gregory, 1973).

Rain drops have a marked effect in removing spores from the air. Collection efficiency is at a maximum with drops of about 2 mm in diameter for all spore sizes; 80 to 90% of spores 20 to 30 microns in diameter will be removed by 2 mm drops (Gregory, 1973).

E. DISCUSSION OF AERIAL TRANSPORT

This report has assumed that the uplifted spores are viable. The viability of the spores was examined by the Agricultural Research Service exotic pest laboratory in Frederick, MD; at least 50% of the teliospores were found to be viable (Bonde et al, 1987).

If the spores that are uplifted during the burning of the wheat fields are viable, it seems likely that spores will at some point in time be transported and deposited in the wheat-growing areas of the United States. The spores are being lifted to altitudes that have wind of sufficient strength and duration to permit long-distance transport. Since the Karnal bunt pathogen has established itself over large areas in northwestern Mexico (the Yaqui and Mayo Valleys of the state of Sonora), a threatening inoculum source is present. It is only a matter of time until favorable conditions for production, liberation, transport, and deposition occur.

Besides being transported by wind and turbulence in moving air masses, Karnal bunt spores may be transported by insects and birds. Smilanick and coworkers (1986) noted that teliospore germination was reduced 46.2% in comparison with the uningested controls by passage through the guts of chickens. Teliospore germination was 70.0% of the controls as a result of grasshopper ingestion. Grasshoppers prefer to feed on bunt-infested kernals than on healthy kernals. Therefore, wide distribution of infested feces by insects and/or birds will enhance the dispersal of the pathogen. The wide dispersal will compensate the pathogen for the reduction in pathogenic potential.

Once in the wheat-growing areas, the establishment of the pathogen is favored by several factors: the longevity of the spores, the use of irrigation, the growing of wheat over large areas, and suitable enviromental conditions.

In soil experiments with teliospores at 0, 3, and 6 inches below the soil, the

maximum length of teliosore survival was 45, 39, and 27 months respectively (Warham, 1986). The longevity of Karnal bunt spores favors the presence of viable spores when suitable host and environmental conditions occur.

The use of irrigation favors development of the disease (Warham, 1986). In the Southwest fields are usually irrigated.

Wheat is extensively grown in southwestern Arizona and in the Imperial Valley of California; 30 to 49 percent of the fields harvested in the Imperial Valley are wheat fields (Bureau of the Census, 1985). It is reasonable to assume that deposition on wheat fields will occur.

The pathogen is present in subtropical dry climatic regions of Iraq and Pakistan. These regions are similar in climate to regions in the southwestern United States.

The probability that a spore will not produce is commonly high; the probablity P(f) of this failure could be similar to the 0.75 and 0.90 which is used in Table 11. However, when multiplication does occur, karnal bunt produces a many-fold increase which is probably close to the calculation for infinity. This decreases the chances of extinction, compensating in part for the high probability of failure. In spite of high failure rates of individual spores, a very small number of spores with a high multiplication rate (similar to infinity) will virtually guarantee establishment (Waggoner, 1962; Table 11).

Table 6. Karnal bunt spores trapped over fields being burnt on the day of burning.

Height		Location			
Feet	(Meters)	Ciudad Obregon	Costa de Hermisillo		Tabasco
10,000	(3,048)	23	2	0	-
5,000	(1,524)	9	0	0	2
1,000	(305)	1	1	0	0

Table 7. Karnal bunt spores trapped near burnt fields on the day after burning.

Height		Location			
Feet	(Meters)	Ciudad Obregon	Costa de Hermisillo		
10,000	(3,048)	0	0	-	0
5,000	(1,524)	1	0	0	0
1,000	(305)	3	0	-	1

Table 8. Terminal velocities (cm/sec) of selected pollens and fungal spores.

Scientific Name	Common Name	Terminal Velocity
[Flowering Plants]		
Alnus viridis	European Green Alder	1.7
Betula alba	Birch	2.4
Pinus sylvestris	Scots Pine	2.5
Quercus robur	English Oak	2.9
[Fungi]		
Erysiphe graminis	Powdery Mildew	1.2
Helminthosporium sativum	Spot Blotch or Foot and Root Rot of Cereals	2.0 - 2.78
Puccinia graminis tritici II	Black Stem Rust of Wheat	0.94 - 1.25
Puccinia graminis tritici I	Black Stem Rust of Wheat	1.06
Puccinia recondita (*Puccinia triticina*)	Leaf Rust of Wheat	1.26
Tilletia caries	Common Bunt of Wheat	1.41
Cronartium ribicola	White Pine Blister Rust	2.03

Table 9. Approximate values of the terminal velocity of Karnal bunt spores.

Assumed Spore Size (microns)	Calculation	Terminal Velocity (cm /sec)
25	(25 X 25)/40	1.56
30	(30 X 30)/40	2.25
35	(35 X 35)/40	3.05
40	(40 X 40)/40	4.00

Table 10. Dimensions in microns of selected fungal spores.

Scientific Name	Common Name	Length X Width
Erysiphe graminis	Powdery Mildew	25-33 X 14-17
Helminthosporium sativum	Spot Blotch or Foot and Root Rot of Cereals	60-120 X 15-20
Puccinia graminis tritici	Black Stem Rust of Wheat	21-42 X 16-22
Puccinia recondita (*Puccinia triticina*)	Leaf Rust of Wheat	16-34 X 13-24
Puccinia striiformis	Stripe Rust of Wheat	25-30 X 12-24
Tilletia caries	Common Bunt of Wheat	14-20 (in dia.)
Tilletia indica (*Neovossia indica*)	Karnal Bunt	23-43 (in dia.)

Table 11. Probability of extinction of a line based on j spores. Multiplication either fails with a probabilirty P(f) or it succeeds and permits an m-fold increase.

Multiplication m-fold	Initial Number of Spores j				
	1	2	4	16	256
			P(f) - 0.75		
or- 4	1.00	1.00	1.00	1.00	1.00
5	0.89	0.79	0.63	0.16	0.01
7	0.80	0.64	0.41	0.03	0.01
Infinity	0.75	0.56	0.32	0.01	0.01
			P(f) - 0.90		
or-10	1.00	1.00	1.00	1.00	1.00
11	0.97	0.94	0.89	0.61	0.01
16	0.92	0.85	0.72	0.26	0.01
Infinity	0.90	0.81	0.65	0.18	0.01

VI. CONCLUSION

If this pathogen is introduced to certain wheat-growing areas , its establishment may be hindered or prevented by various factors.

One factor is crop rotation. If wheat is infrequently grown in the crop rotation, adverse climatic factors may reduce the number of teliospores. The crop rotations near the Mississippi Valley may hinder or prevent disease development.

A second factor is freezing temperatures. Prolonged cold periods along with high soil moisture, the conditions that prevail in the upper Midwest and the inland Pacific Northwest during winter, will hinder or prevent disease development by preventing teliospores from overwintering.

A third factor is cultural practices. For example, moldboard plowing will affect the ability of teliospores to survive and infect. Buried teliospores are unable to produce sporidia on the soil surface; in addition, buried teliospores, according to some researchers, have shorter survival periods.

A fourth factor is soil moisture. There appears to be a negative correlation between teliospore survival and soil moisture. Because of this possible soil moisture factor, disease development in a warm-temperate (V) climatic zone, such as the lower Mississippi Valley, or a typical-temperate (VI) climatic zone, such as the Midwest, may be hindered or prevented. There is no record of karnal bunt in a warm-temperate or typical-temperate climatic zone; however, the pathogen may be able to flourish in these zones, but has not been introduced.

In spite of pathogen-hindering factors in some areas of the United States, there are large wheat-growing areas in the Southwest that appear to be favorable environments for the establishment of the karnal bunt pathogen. These areas are:

A. The white wheat area of Arizona;

B. The white wheat area of California; and

C. The hard and soft red winter area of New Mexico and Texas.

The white wheat areas of Arizona and California are very close to infested areas in northwestern Mexico. Transport of the Karnal bunt pathogen is extremely likely.

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ADDENDUM 1

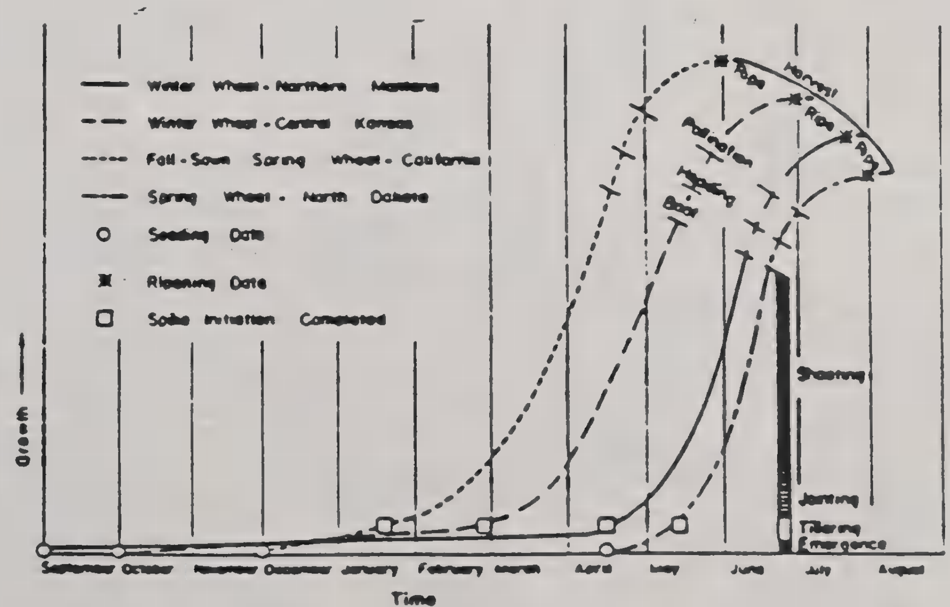
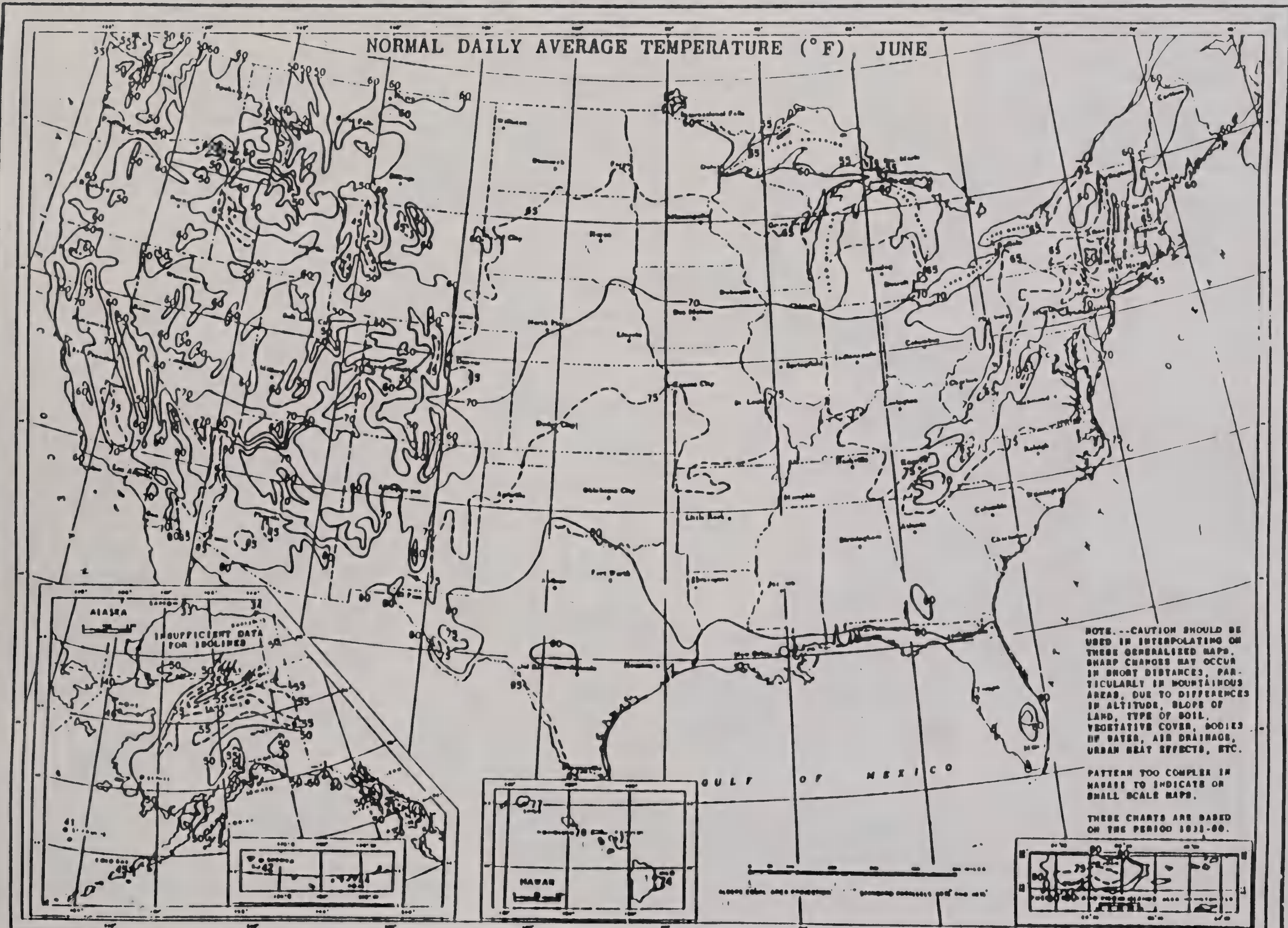


Fig. 3.—Approximate growth cycles of wheat plants when sown on different dates. (Courtesy U.S. Department of Agriculture)

NORMAL DAILY AVERAGE TEMPERATURE (°F) APRIL
NOTE.--CAUTION SHOULD BE USED IN INTERPOLATING ON THESE GENERALIZED MAPS. SHARP CHANGES MAY OCCUR IN SHORT DISTANCES, PARTICULARLY MOUNTAINOUS AREAS, DUE TO DIFFERENCES ALTITUDE, SLOPE OF LAND, TYPE OF SOIL, VEGETATIVE COVER, BODIES WATER, AIR DRAINAGE, URBAN HEAT EFFECTS, ETC.
PATTERN TOO COMPLEX IN HAWAII TO INDICATE ON SMALL SCALE MAPS.
THESE CHARTS ARE BASED ON THE PERIOD 1931-60.
INSUFFICIENT DATA FOR ISOLINES
ALASKA
HAWAII
GULF OF MEXICO


NORMAL DAILY AVERAGE TEMPERATURE (°F), MAY
ALASKA
INSUFFICIENT DATA FOR ISOLINES
HAWAII
GULF OF MEXICO
NOTE.--CAUTION SHOULD BE USED IN INTERPOLATING ON THESE GENERALIZED MAPS. SHARP CHANGES MAY OCCUR IN SHORT DISTANCES, PARTICULARLY IN MOUNTAINOUS AREAS, DUE TO DIFFERENCES IN ALTITUDE, SLOPE OF LAND, TYPE OF SOIL, VEGETATIVE COVER, BODIES OF WATER, AIR DRAINAGE, URBAN HEAT EFFECTS, ETC.
PATTERN TOO COMPLEX IN HAWAII TO INDICATE ON SMALL SCALE MAPS.
THESE CHARTS ARE BASED ON THE PERIOD 1931-60.


NORMAL DAILY AVERAGE TEMPERATURE (°F) JUNE
ALASKA
INSUFFICIENT DATA FOR ISOLINES
HAWAII
GULF OF MEXICO
NOTE.--CAUTION SHOULD BE USED IN INTERPOLATING ON THESE GENERALIZED MAPS. SHARP CHANGES MAY OCCUR IN SHORT DISTANCES, PARTICULARLY IN MOUNTAINOUS AREAS, DUE TO DIFFERENCES IN ALTITUDE, SLOPE OF LAND, TYPE OF SOIL, VEGETATIVE COVER, BODIES OF WATER, AIR DRAINAGE, URBAN HEAT EFFECTS, ETC.
PATTERN TOO COMPLEX IN HAWAII TO INDICATE ON SMALL SCALE MAPS.
THESE CHARTS ARE BASED ON THE PERIOD 1931-60.

ADDENDUM 3

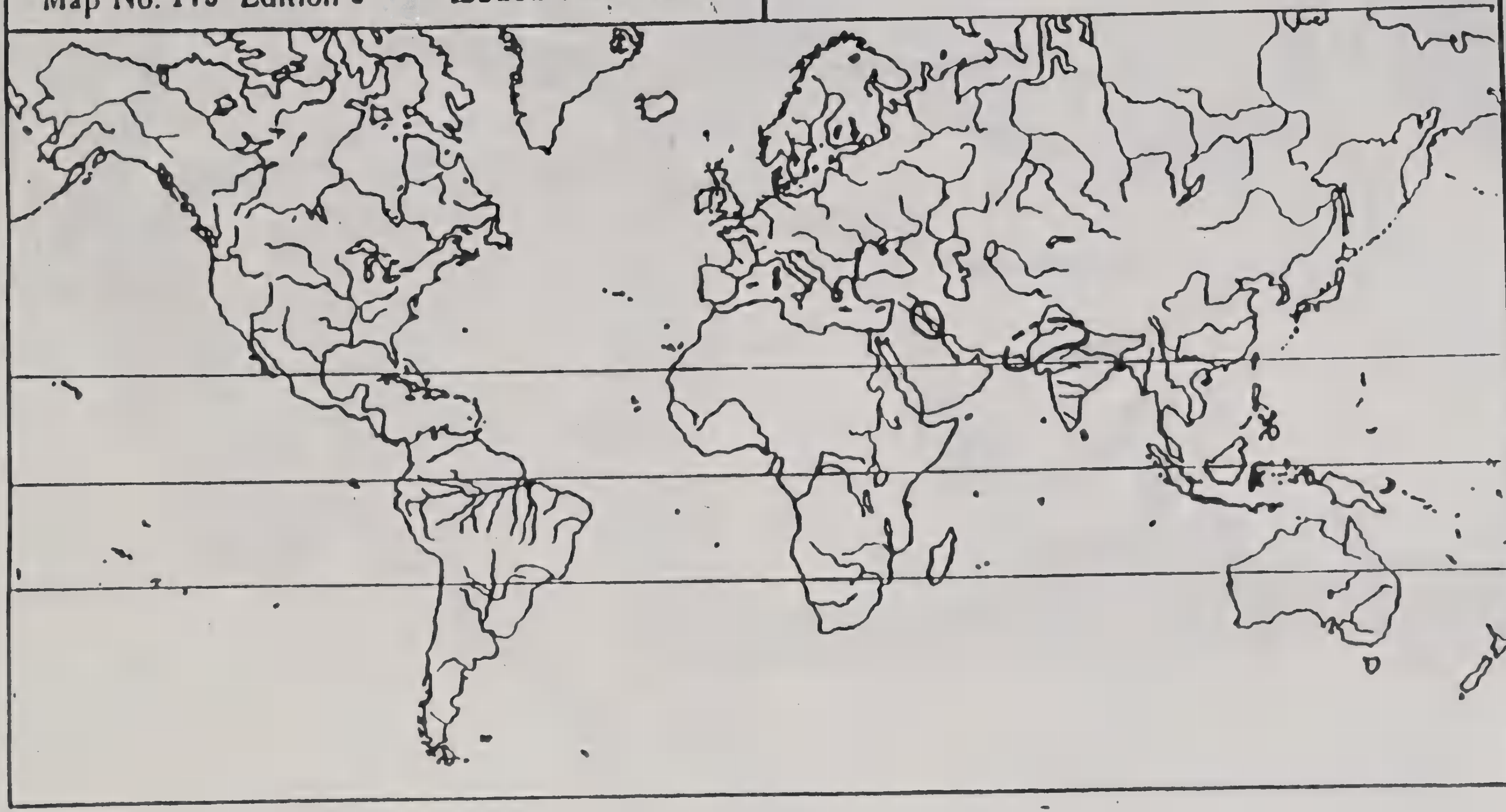
COMMONWEALTH MYCOLOGICAL INSTITUTE

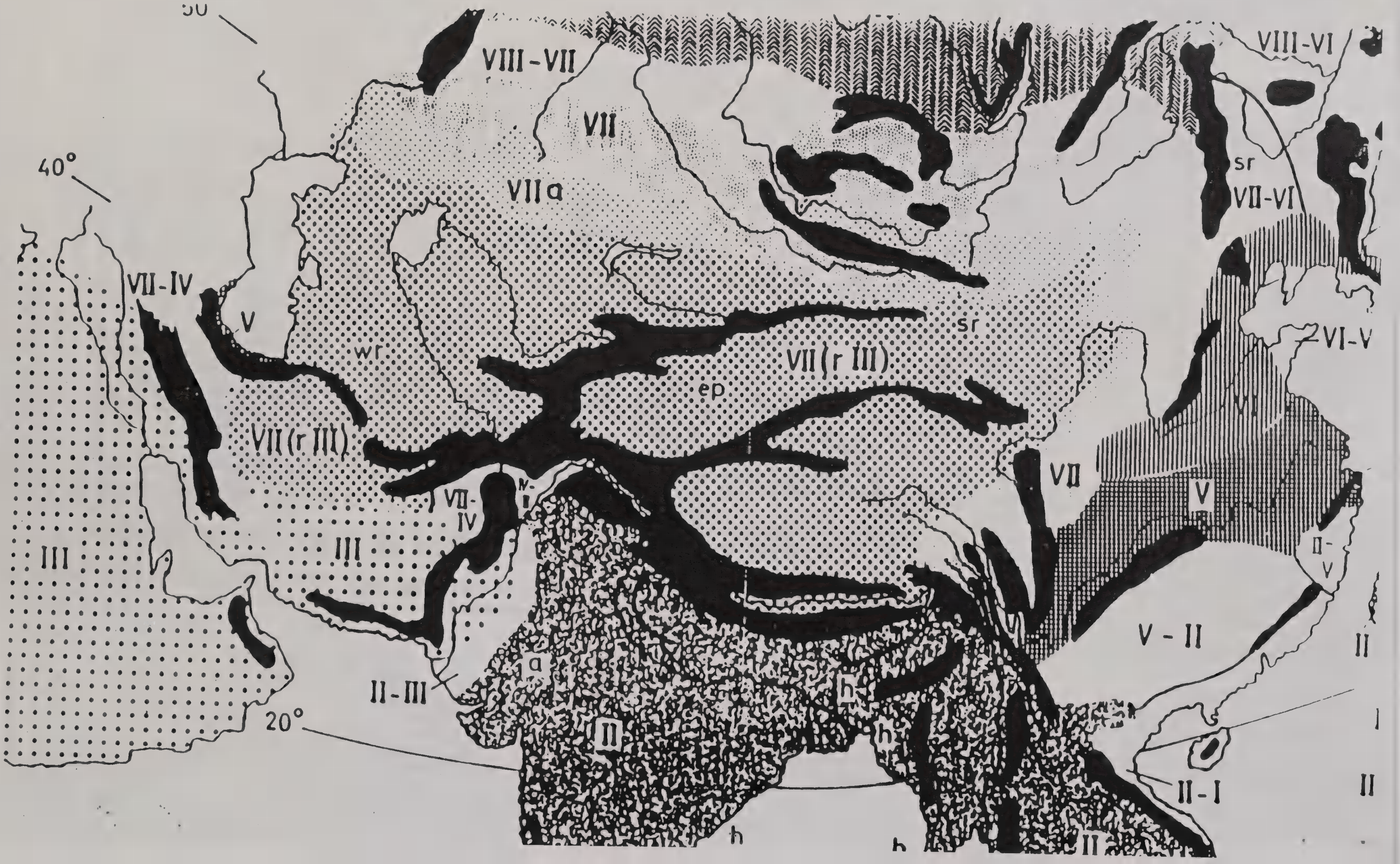
DISTRIBUTION MAPS OF PLANT DISEASES

Map No. 173 Edition 3 Issued 1. iv. 1974

Pathogen: Tilletia indica Mitra

Hosts: Wheat (Triticum)

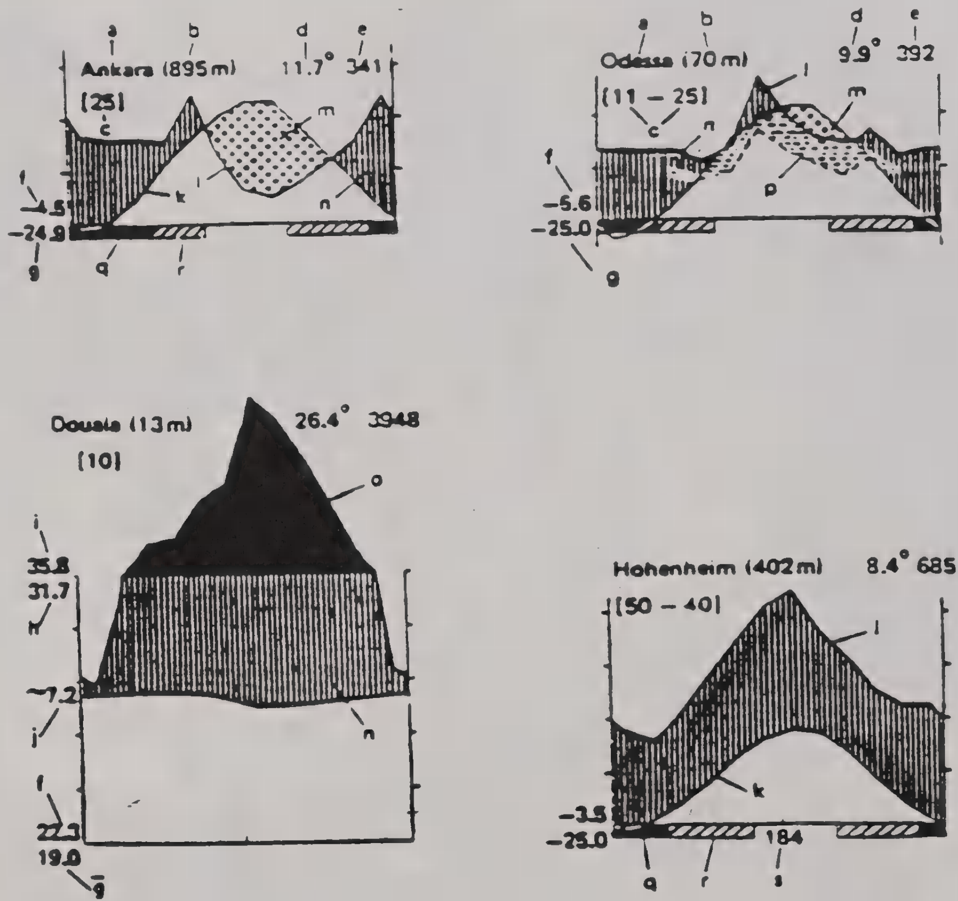




VIII-VII
VII
VIIa
VIII-VI
sr
VII-VI
40°
VII-IV
V
wr
sr
VII (r III)
ep
VI-V
VII (r III)
VII
VII-IV
V
III
III
II-V
V-II
II
II-III
a
h
20°
II
h
II-I
II
h
h

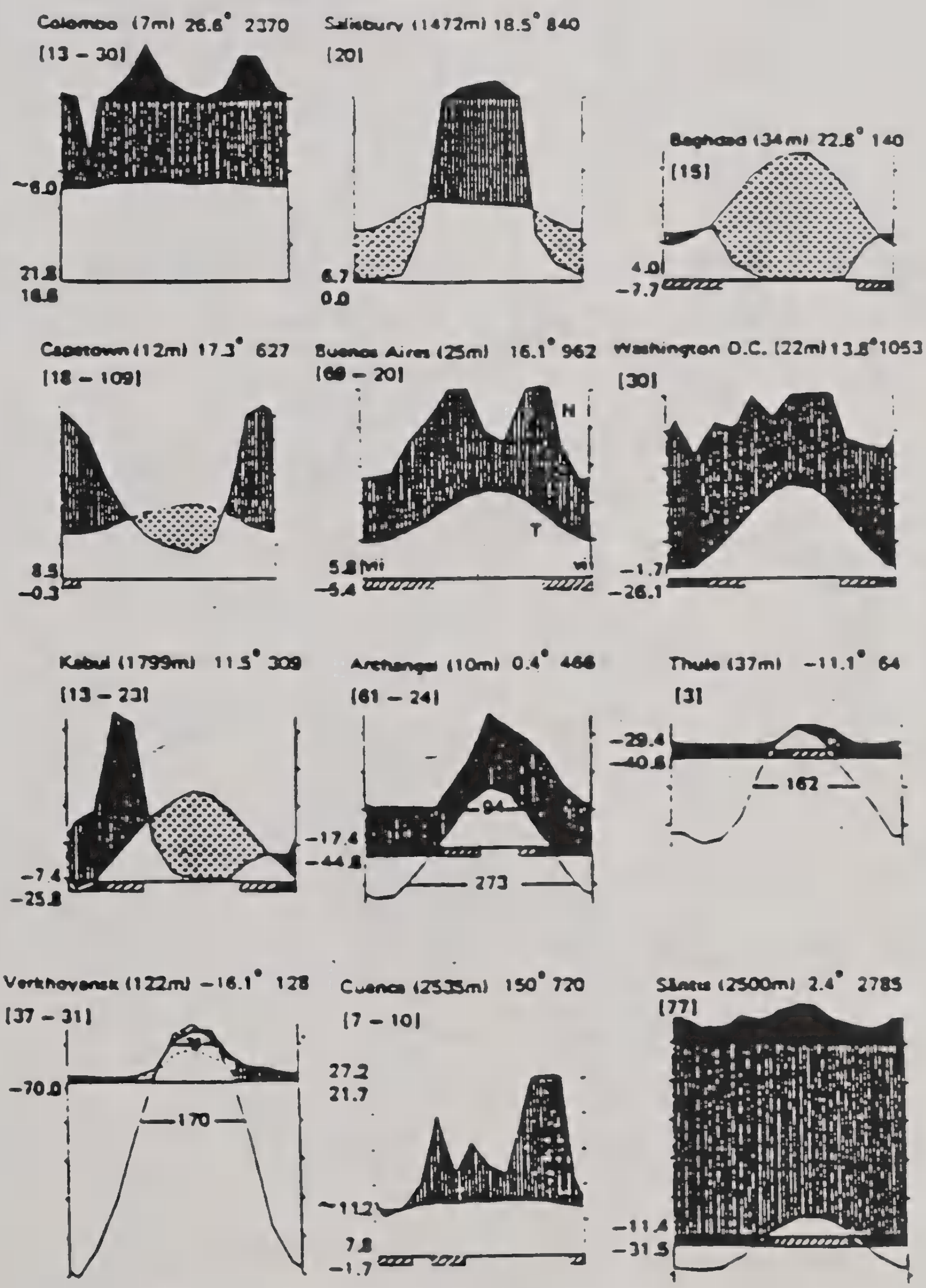
ADDENDUM 5


70°
60°
50°
40°
30°
20°
IX
IX
VIII - IX
VIII - IX
VIIa
VIII
VIII - VII
co
VIII
VIII - VI
VII - VI
VIIa
VII
co
VI
V - VI
V
VII - V
V - II
II - V
V - III
II - III
II - III
III
sr
sr
III
II
II
II - I
II - I
II
II
V - IV
IV - III
wrs
swr
wr
h
V
a



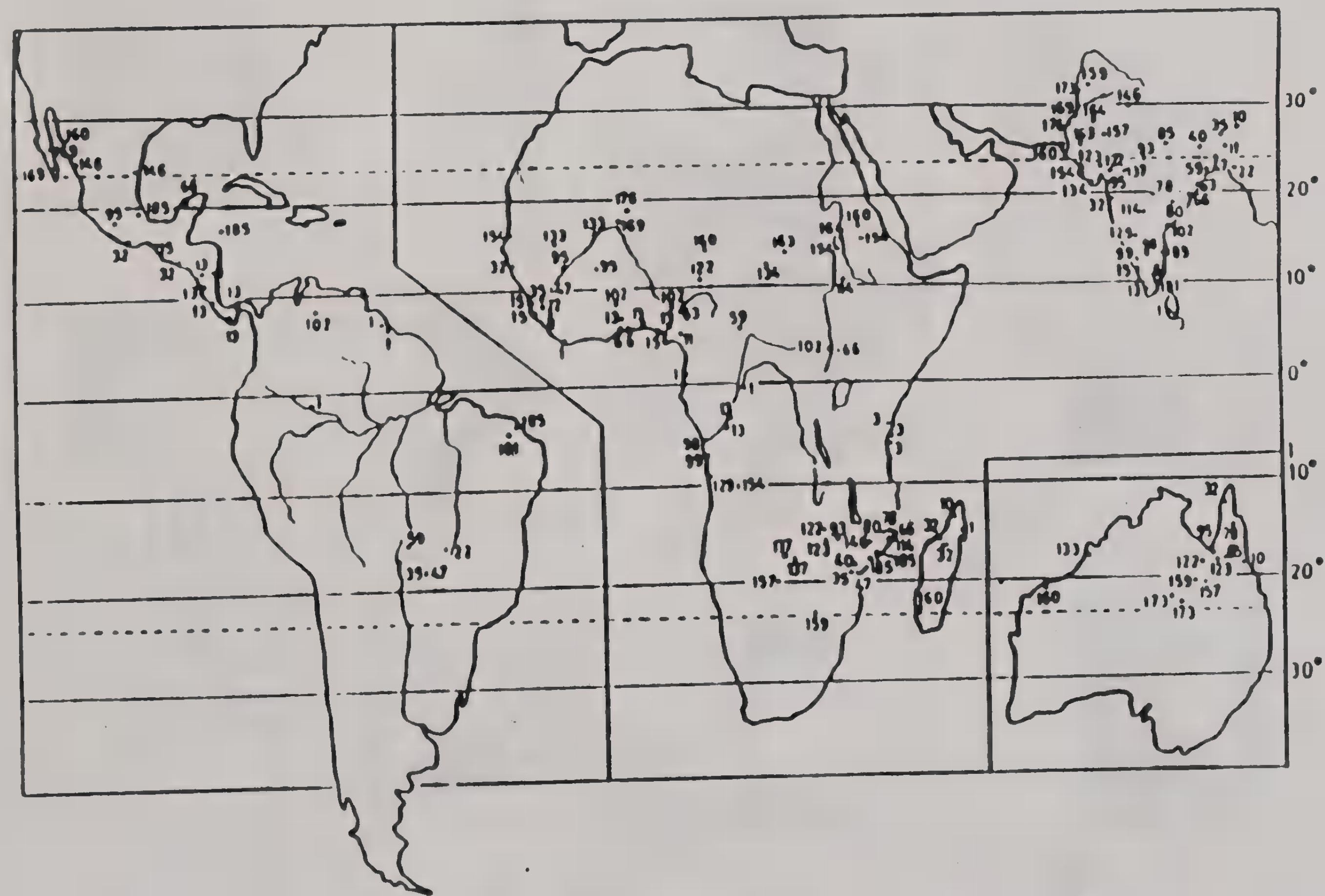
Key to the climatic diagrams. Abscissa: Months (N. Hemisphere January—December, S. Hemisphere Juli—June); Ordinate: one division = 10° C or 20 mm rain. a = station, b = height above sealevel, c = duration of observations in years (of two figures the first indicates temperature, the second precipitation), d = mean annual temperature in ° C, e = mean annual precipitation in mm, f = mean daily minimum of the coldest month, g = lowest temperature recorded, h = mean daily maximum of the warmest month, i = highest temperature recorded, j = mean daily temperature variations, k = curve of mean monthly temperature, l = curve of mean monthly precipitation, m = relative period of drought (dotted), n = relative humid season (vertical shading), o = mean monthly rain $>$ 100 mm (black scale reduced to 1/10), p = reduced supplementary precipitation curve (10° C = 30 mm) and above it (dashes) dry period, q = months with mean daily minimum below 0° C (black) = cold season, r = months with absolute minimum below 0° C (diagonal shading) = late or early frosts occur, s = mean duration of frost-free period in days. Some values are mising, where no data are available for the stations concerned (h—j are only given for diurnal types of climate).

ADDENDUM 7



Typical climatic diagrams for the climatic zones I—X. I Colombo, II Salisbury, III Baghdad, IV Cape Town, V Buenos Aires, VI Washington (see also Fig. 7 Hohenheim near Stuttgart), VII Kabul (see also Fig. 7, Ankara and Odessa), VIII Archangel, IX Thule (Iceland), VIII dry (IX) Verchojansk (Siberia), X (I—II) Cuenca in Ecuador, X (V) Säntis (Alps).

ADDENDUM 8

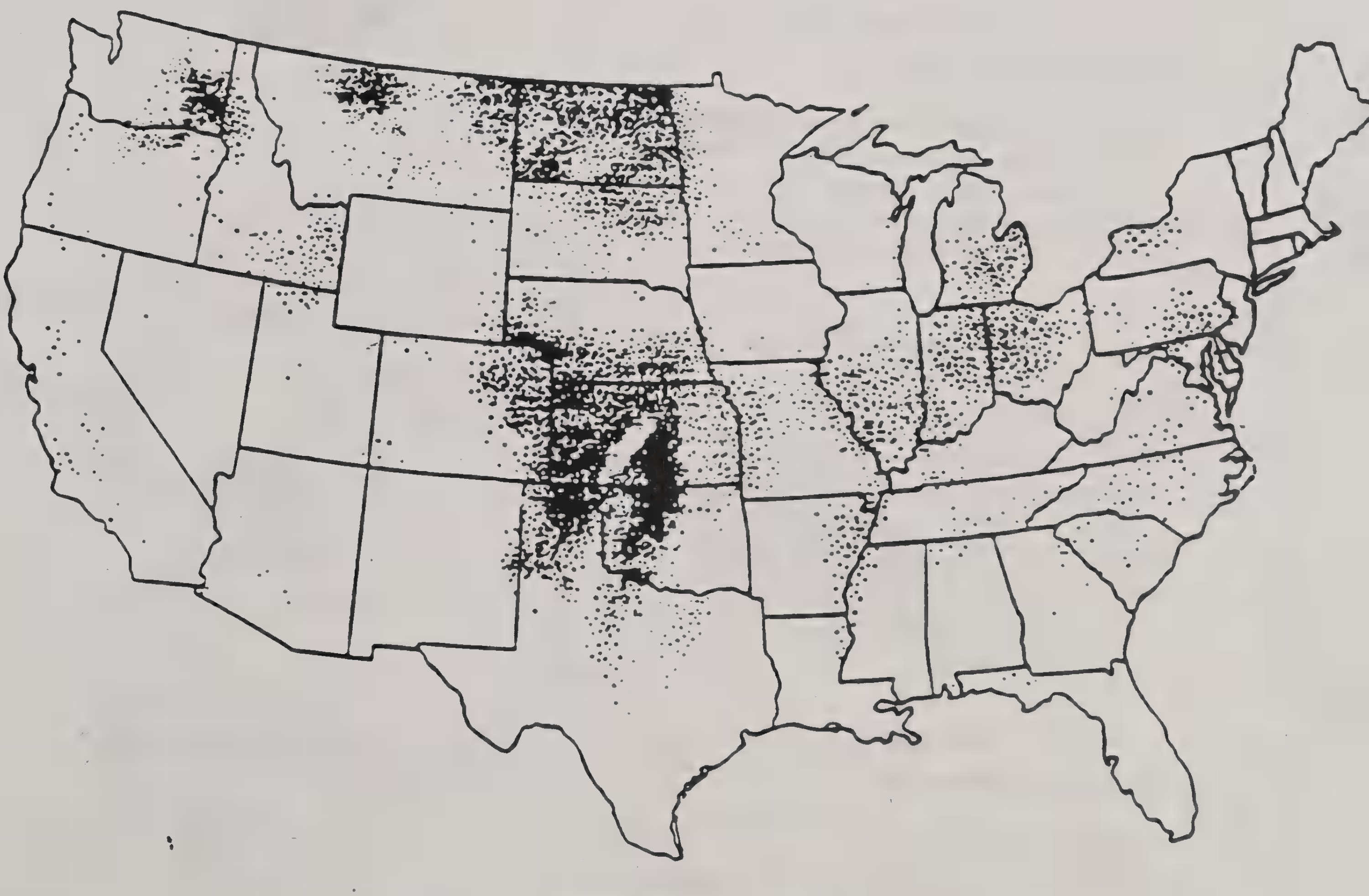


The Indian stations bear the same number as that used in the Climatic diagram-World-Atlas. The same number indicates their homoclimes in other parts of the world (from Walter, a UNESCO project).

ADDENDUM 9

Where wheat is grown in the United States

(A composite of all types of wheat)



Each dot represents 10,000 acres

ADDENDUM 10

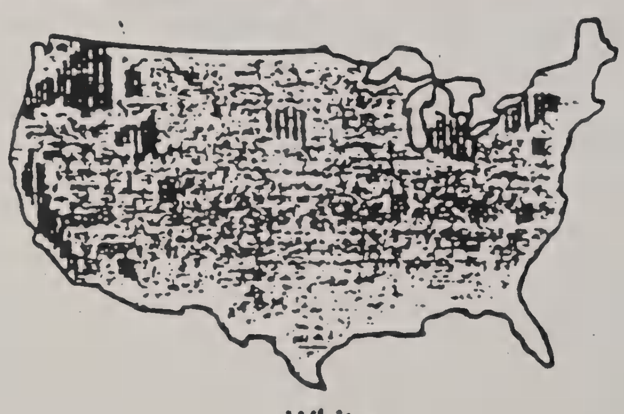
Where different kinds of wheat are grown in the United States

The map indicates the general areas in which the various kinds of wheat are grown. The classes of wheat grown in an area are determined by climate, soil, rainfall and irrigation.

Hard Red Spring

Soft Red Winter

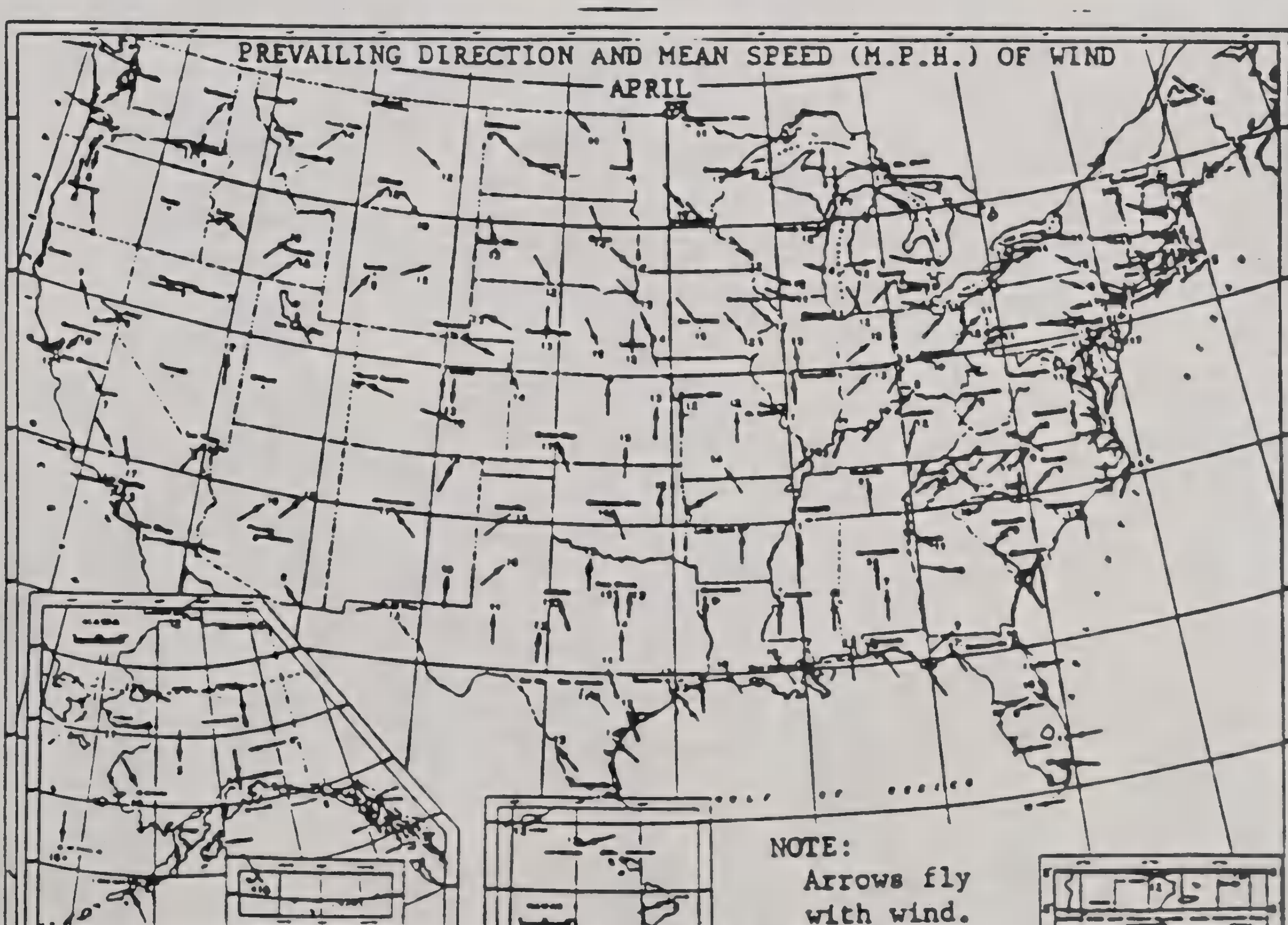
Hard Red Winter

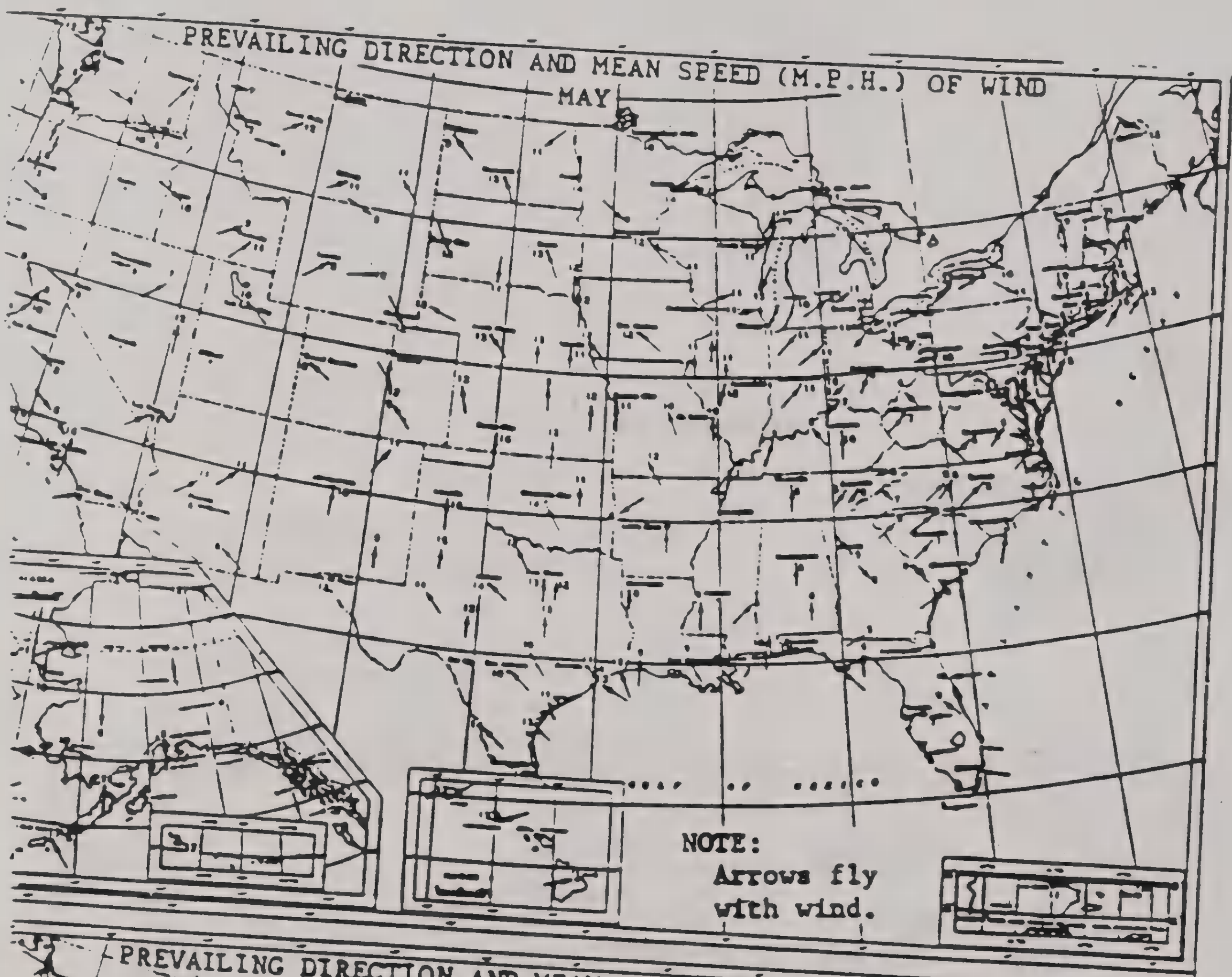


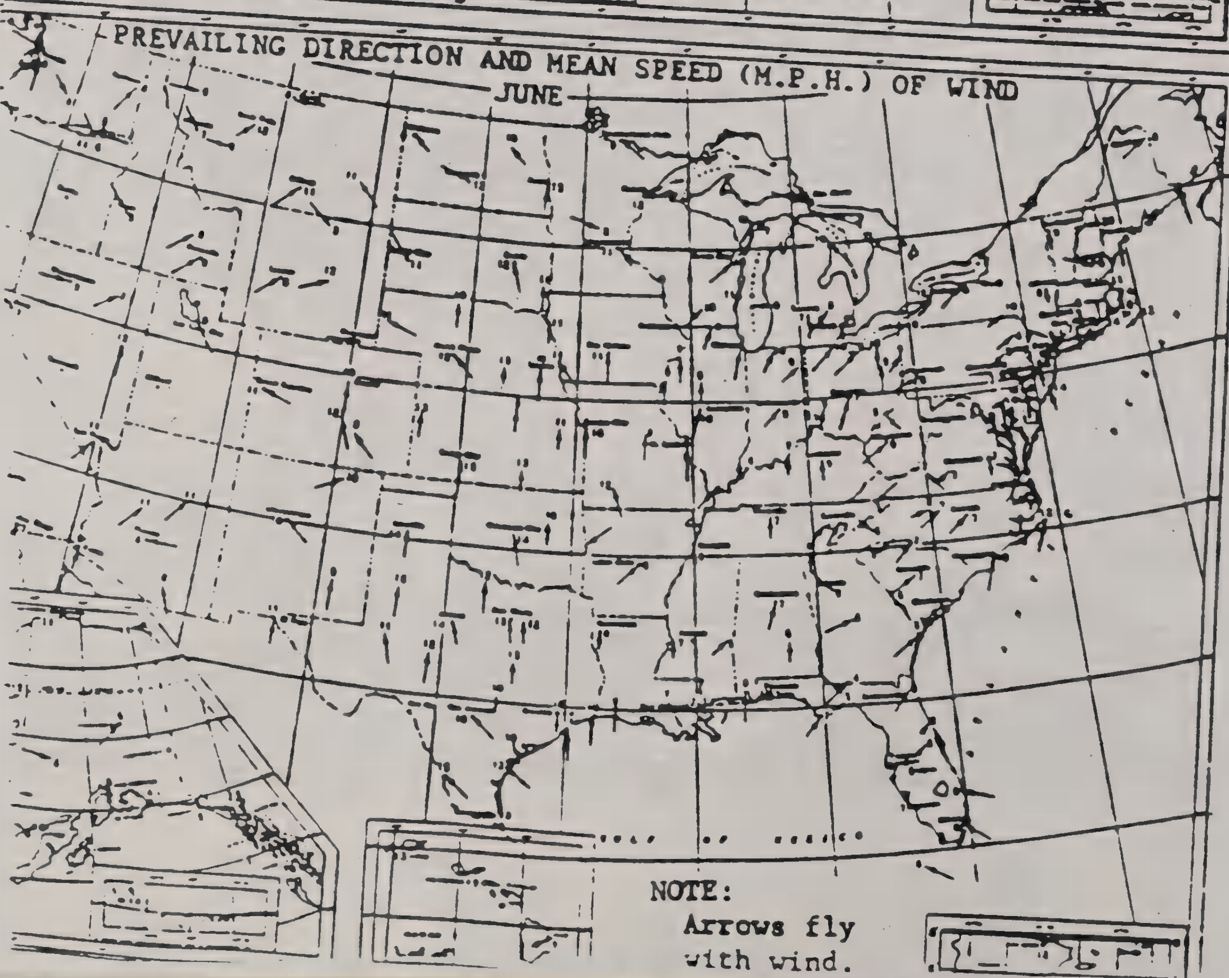
White

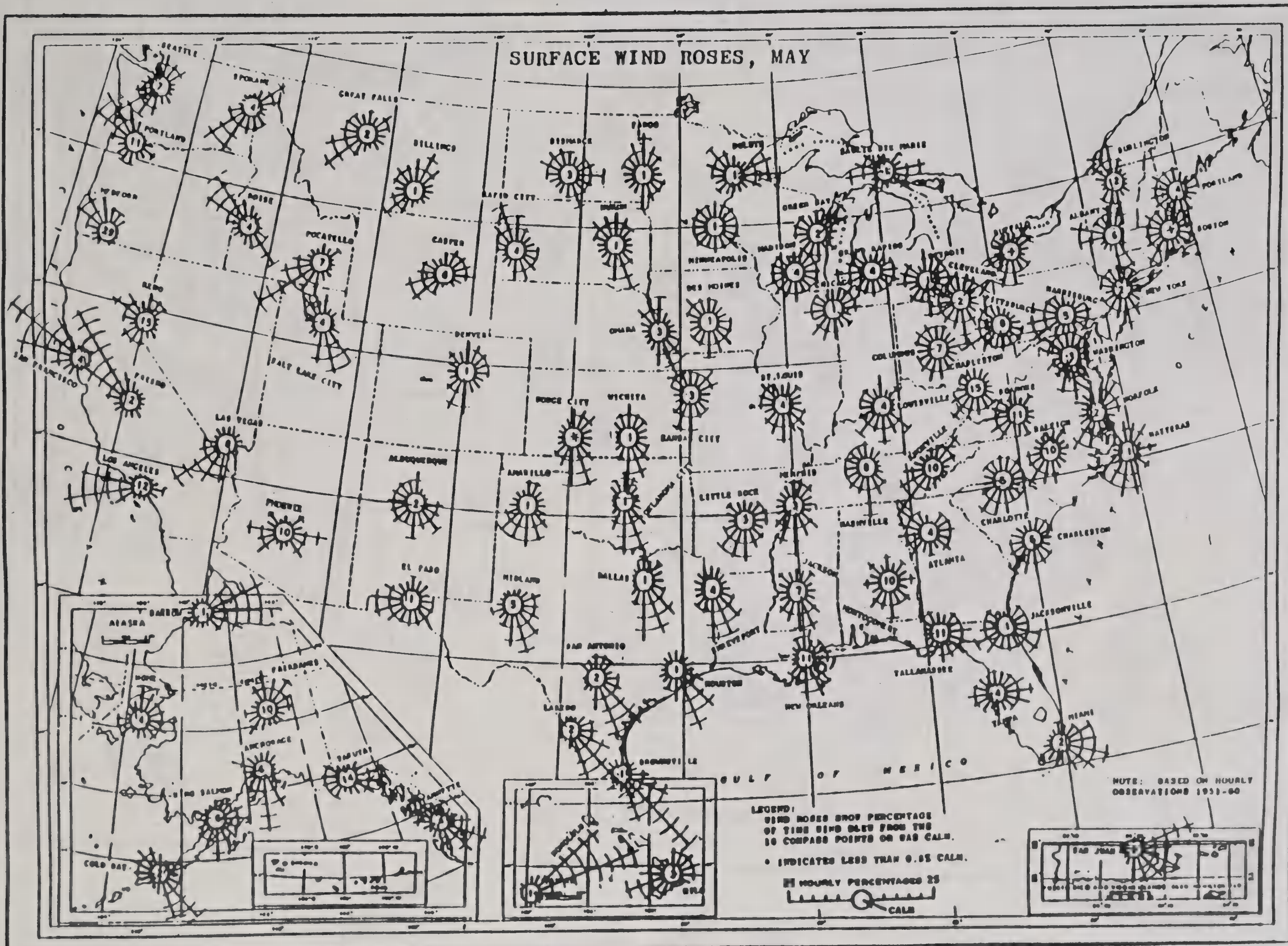
Durum

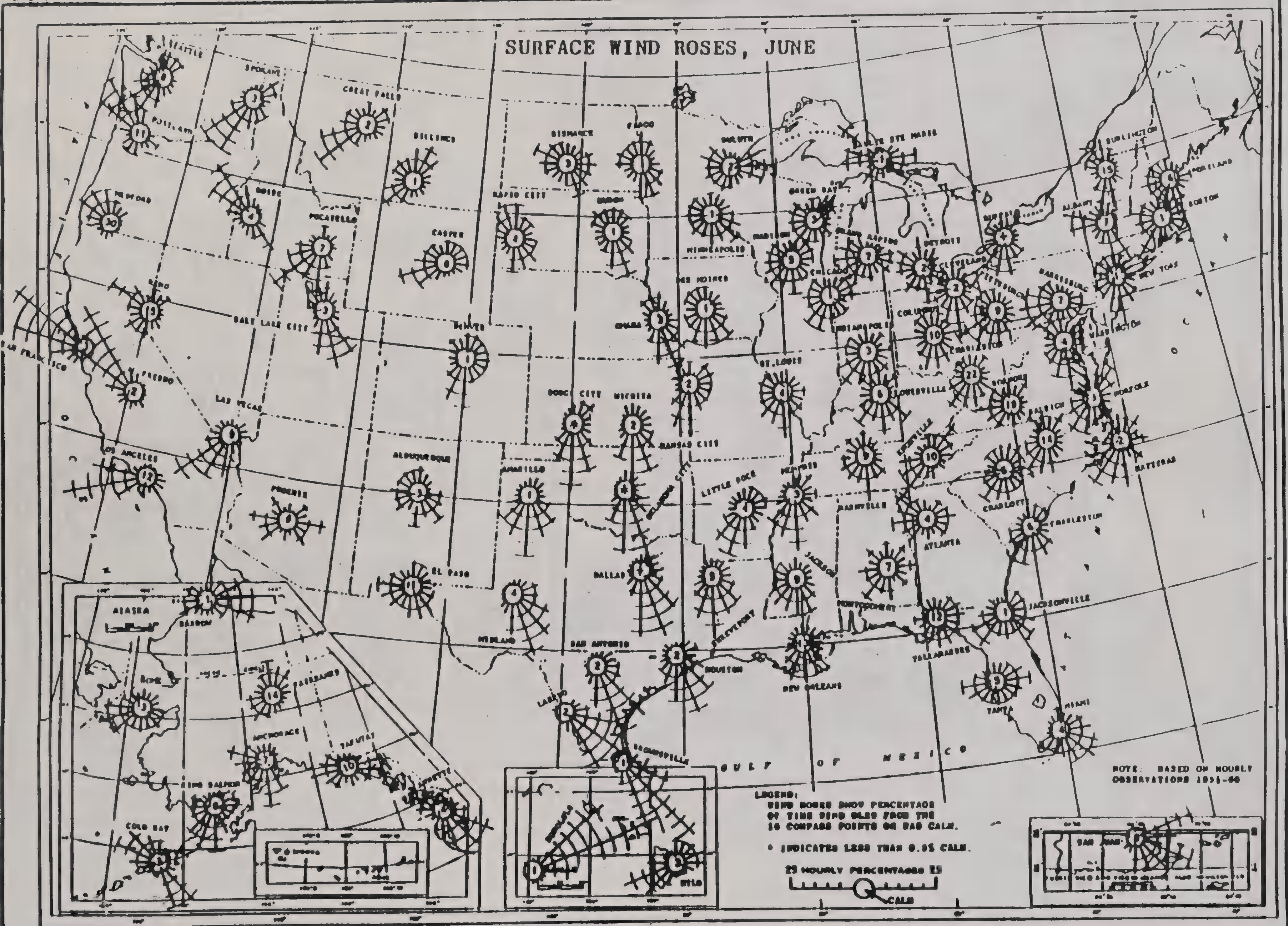
ADDENDUM 11


PREVAILING DIRECTION AND MEAN SPEED (M.P.H.) OF WIND
APRIL
NOTE:
Arrows fly
with wind.


PREVAILING DIRECTION AND MEAN SPEED (M.P.H.) OF WIND
MAY
NOTE:
Arrows fly with wind.


PREVAILING DIRECTION AND MEAN SPEED (M.P.H.) OF WIND
JUNE
NOTE:
Arrows fly with wind.


SURFACE WIND ROSES, MAY
NOTE: BASED ON HOURLY OBSERVATIONS 1951-60
LEGEND:
WIND ROSES SHOW PERCENTAGE OF TIME WIND BLEW FROM THE 16 COMPASS POINTS OR WAS CALM.
• INDICATES LESS THAN 0.5% CALM.
HOURLY PERCENTAGES 25
CALM
GULF OF MEXICO
SEATTLE
SPOKANE
PORTLAND
GREAT FALLS
BILLINGS
BISMARCK
FARGO
DULUTH
BURLINGTON
PORTLAND
BOISE
POCATELLO
CASPER
RAPID CITY
MINNEAPOLIS
GREEN BAY
MADISON
ALBANY
BOSTON
BUFFALO
DETROIT
CLEVELAND
NEW YORK
RENO
SALT LAKE CITY
DENVER
OMAHA
DES MOINES
COLUMBUS
PITTSBURGH
HARRISBURG
WASHINGTON
SAN FRANCISCO
FRESNO
LAS VEGAS
DODGE CITY
WICHITA
KANSAS CITY
ST. LOUIS
LOUISVILLE
CHARLESTON
NORFOLK
HATTERAS
RALEIGH
LOS ANGELES
ALBUQUERQUE
AMARILLO
LITTLE ROCK
MEMPHIS
NASHVILLE
CHARLOTTE
CHARLESTON
ATLANTA
PHOENIX
EL PASO
MIDLAND
DALLAS
JACKSON
MONTGOMERY
JACKSONVILLE
SHREVEPORT
SAN ANTONIO
HOUSTON
NEW ORLEANS
TALLAHASSEE
TAMPA
MIAMI
LAREDO
BROWNSVILLE
ALASKA
BARROW
NOME
FAIRBANKS
ANCHORAGE
YAKUTAT
KING SALMON
COLD BAY
SAN JUAN


SURFACE WIND ROSES, JUNE
NOTE: BASED ON HOURLY OBSERVATIONS 1951-60
LEGEND:
WIND ROSES SHOW PERCENTAGE OF TIME WIND BLEW FROM THE 16 COMPASS POINTS OR WAS CALM.
0 INDICATES LESS THAN 0.5% CALM.
25 HOURLY PERCENTAGES 25
CALM
GULF OF MEXICO
ALASKA

PEST RISK ANALYSIS ON KARNAL BUNT

PEST: Tilletia indica (Neovossia indica)

DISEASE: Karnal Bunt (Partial Bunt)

ANALYST: Robert A. Schall

FILE NO.: 1

COMMODITY: Wheat - Rye

DATE: November 4, 1991

DOMESTIC CONCERN: Wheat - Rye

ORIGIN: Possible Introduction

Reviewers:

Sue Cohen, Plant Pathologist, PPD-PRAS
Robert Griffin, Plant Pathologist, PPQ-PD
Bud Petit De Mange, Operations Officer, PPQ-PO
Stephen Poe, Plant Pathologist, PPQ-OS
Matt Royer, Chief Operations Officer, Plant Pathologist, PPQ-BATS
Arnold Tschanz, Senior Staff Officer, PPQ-BATS

CONTENTS:

I. SUMMARY OF LIFE CYCLE, DISTRIBUTION, AND EPIDEMIOLOGY

Teliospores, the thick-walled overwintering spores, persist in soil and in or on kernels. Seed contaminated with teliospores can introduced the pathogen to new areas. In response to moisture in the spring, the teliospores germinate and develop spore-bearing structures (promycelia). If cool, wet weather occurs during flowering, air currents and splashing water will carry thin-walled spores (primary and secondary sporidia) to the host plants to initiate infections. The infections lead to partial or complete conversion of developing kernels into darkened masses of teliospores. At harvest bunted kernels liberate spores that contaminate soil and seed (Wiese, 1977; Warham, 1986).

II. HOSTS

The following are hosts.

Scientific Name	Common Name	Reference
Triticum aestivum	Wheat	Joshi et al, 1983
Triticum durum	Durum wheat	"
Triticale hexaploide	Triticale	"
Secale cereale	Rye	Warham, 1988
Triticum spp.	Wild species of wheat	Singh, 1986
Aegilops spp.	Goatgrass	Warham et al, 1986

III. DISSEMINATION/VECTORS

Literature:

According to Smilanick and coworkers (1986), germination of teliospores ingested by leghorn chickens was 46% of uningested controls; germination of teliospores ingested by grasshoppers, Melanoplus sanguinipes, was 70% of uningested controls. Although reduced by ingestion by potential vectors, teliospore germination remained at a high level.

After burning of infected wheat fields, viable teliospores were collected below and at the 3,000 m altitude (Bonde, 1987); it is possible that these teliospores will be transported several hundred miles (Schall, 1988).

Discussion:

Viable teliospores could be widely disseminated by the natural movement of grain-eating migratory animals and by the importation and use of bunt-contaminated, animal-derived fertilizers. It is

a common practice to feed severely bunt-contaminated grain to feedlot animals; in this case, application of manure from the feedlot animals would deposit teliospores on the soil surface in a manner conducive to natural infection of wheat. In addition, teliospores could also be transported over long distances by air currents. Finally, teliospore-contaminated harvesting equipment, barges, and transportation equipment could transport the pathogen.

IV. RATING ELEMENTS OF RISK MODEL

PROBABILITY OF ESTABLISHMENT

Estimate probability of pest being on, with, or in the plant commodity during import. <u>High</u>

<u>Literature:</u>

Where the pathogen is established in favorable areas in India, there is a high prevalence of infected samples in favorable years; for example, the percentage of infested wheat samples from threshing floors was 93.1% in the State of Punjab in 1980 (Singh et al, 1983). In Mexico in the Mayo and Yaqui Valleys where the pathogen was introduced in the 70's, 6% and 64% of the samples collected were infected in 1982 and 1983, respectively (Warham, 1986).

Newly-formed teliospores generally have a dormant period of 6-8 months (Zhang et al, 1984). Afterwards, if under suitable conditions, the teliospores will be capable of germinating for years. When bunted grains were stored in a laboratory refrigerator at 10 C, the teliospores survived for more than 5 years. At the 5-year mark, germination was approximately 4%. When bunted grains were stored at room temperature (17-33 C), the teliospores retained their viability for 4.5 years. At the 4.5-year mark, germination was approximately 2% (Krishna and Singh, 1983).

<u>Discussion:</u>

Where Karnal bunt occurs, there will be years in which a high percentage of the grain will be infected. Therefore, because of (1) infection of kernels in the field, (2) contamination of uninfected kernels during harvest, and (3) contamination of pathogen-free grain in contaminated grain-handling facilities, the grain from infested regions is likely to be contaminated.

If the grain is contaminated, teliospores are likely to survive for long periods under typical storage conditions.

Estimate probability of pest surviving in transit and successfully passing undetected at ports of entry. High

Literature:

If at 10 C or at room temperature, the teliospores will survive transit easily (Krishna and Singh, 1983).

Teliospores can survive 10 weeks of deep freezing (-18 C), although germination is almost totally inhibited. One week of deep freezing had no adverse effect; 2 weeks decreased the germination by half (Zhang et al, 1984). Scientists at the USDA Foreign Disease - Weed Science Research Unit in Frederick, MD found that freezing for two months did not significantly inhibit germination (Matt Royer - personnel communication).

Some kernels are completely infected, but most are partially infected; therefore, visual examination may not detect a low-level presence (Warham, 1990).

Only specialized tests, such as the glycerol filtration seed assay, will detect trace amounts of teliospores contaminating wheat seed (Matsumoto and Bell, 1969; Warham, 1986).

Discussion:

The teliospores are capable of surviving the temperature extremes usually encountered during transit.

Partially infected kernels, particularly if few in number, could easily escape detection.

Although fully and partially bunted kernels, if present in significant numbers, could be detectable by visual inspection, kernels contaminated with trace amounts of teliospores would probably pass visual inspection at ports of entry. Only specialized tests, such as the glycerol filtration seed assay, will detect trace amounts of teliospores in wheat seed.

Estimate probability of pest successfully colonizing. High

Literature:

First found in the State of Haryana, India, the disease is present across northern India from the western border to Bihar and West Bengal. The KB pathogen also occurs in Afghanistan, Iraq, Pakistan, Mexico, and Nepal (Joshi et al, 1983; Royer and Rytter, 1985; Singh and Agaral, 1989). The occurrence in Afghanistan is not mentioned in some publications (Waller and Mordue, 1983). Lambat (1983) considers the pathogen to be established in Lebanon, Syria, and Turkey in the Middle East; however, this is based on the examination of wheat germplasm seed packets.

Walter (1973) divides the world into nine main climate zones. The KB pathogen is established in several main climate zones and transitional zones.

Climate Zone	Title	Climate Zone Characteristics	Geographical Area
II.	Tropical	Rainy season in the summer; dry season in the cool months	India
III.	Subtropical Dry	Rainfall is very low; daytime temperatures are very high	Iraq, N. Mexico
II-III.	Trans. Zone		S. Pakistan
IV-II.	Trans. Zone		N. Pakistan
VII-IV.	Trans. Zone		Afghanistan

Based on the ability of the teliospores to survive freezing temperatures (Smilanick et al, 1985; Zhang et al, 1984), it is probable that the KB pathogen can survive in at least the Warm Temperate (V) and possibly the Typical Temperate (VI) Zone.

V.	Warm Temperate	Scarcely any or no winter, extremely wet, esp. in summer	Southeastern U. S.
VI.	Typical Temperate	Winters are cold but not long, summers are cool when oceanic	Northeastern U. S.
VII.	Arid Temperate	Continental climate; little precipitation	Great Plains